BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

REMARKS

Upon entry of the present amendment, claims 1-36 and 41-47 are pending in this application.

Also submitted with this response is an Information Disclosure Statement for consideration by the Examiner, and the appropriate fee under 37 CFR §1.17(p).

Applicants note with appreciation the recognition that claims 8-16, 18, 20, 21, 23, and 25-36 are patentable over the art of record.

Claims 37-40 are cancelled without prejudice or disclaimer. Claims 1-12 and 41-47 have been amended to clarify the subject matter Applicants consider the claimed invention. Support for the amended claims is found throughout the application and claims as originally filed, *see, e.g.,* claims 17, 22, 27, and 32, and Figure(s) 3, 14, 39, and 44. No new matter is added. In particular, the terms "phenolic" and/or "moiety" have been deleted from claims 1-7, 10, and 12 and replaced with the new terms "hydroxyl" or "group". The new terms are respectfully submitted to be more precise and more in keeping with Applicants' intent and disclosure. Therefore, it is clearer that the claims, as amended herein, include within their scope and distinctly point out trimethoxyphenyl-substituted indole ligands that are substituted further with one or more hydroxyl functional groups (*i.e.,* –OH), rather than a phenolic group (*i.e.,* Ar-OH).

The Examiner's rejections and objections are addressed in turn as set forth in the Office Action.

RESTRICTION UNDER 35 U.S.C §121 AND 35 U.S.C §372

Applicants affirm the provisional election that was made during a telephonic interview with the examiner on September 8, 2003. Applicants wish to prosecute the invention of Group I, Claims 1-36 and 41-47, and withdraw their traversal. Claims 37-40 have been cancelled without prejudice or disclaimer. Applicants reserve the option to prosecute any of the cancelled subject matter in a future divisional patent application(s).

OBJECTION TO SPECIFICATION UNDER 37 CFR 1.72(b)

Applicants hereby submit an Abstract as an attachment to this response in order to comply with the requirements of 37 CFR 1.72(b).

REJECTION UNDER 35 U.S.C §112, SECOND PARAGRAPH

Claims 2, 7 and 41-47 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter Applicants regard as the invention. Claim 2 has been amended to include a closed parenthesis, in accordance with the Examiner's suggestion. Claim 7 has been amended to delete the duplicate parenthesis and the duplicate phrase "may be the same or different".

In claims 41, 42, 44, and 45, the Examiner considered the phrase "tubulin-containing system" to be unclear. In order to overcome this rejection, the amended claims now recite that the inhibition of tubulin polymerization takes place in a cell.

Claims 41, 43, 46, and 47 have been amended to insert the phrase "one" between any and of Claims 1-36, in accordance with the Examiner's suggestion.

Claim 43 has been amended to a) replace the phrase "host" with "mammal" and b) insert the phrase "therapeutically effective amount of", in accordance with the Examiner's suggestion.

Claim 45 has been amended to replace the phrase "may be chosen from the group containing" with "is selected from the group consisting of", in accordance with the Examiner's suggestion.

Claim 46 has been amended to replace the phrase "A preparation for pharmaceutical use containing" with "A pharmaceutical composition comprising", in accordance with the Examiner's suggestion.

The Examiner considered Claim 47 to be unclear as to what is being administered or where the tumor vasculature is being destroyed. Claim 47 has amended to distinctly point out that tumor vasculature destroyed is located in a patient and that any of the compounds of claims 1-36 may be administered to destroy the tumor vasculature located in a patient.

Applicants respectfully submit that the above amendments and comments obviate the rejection under 35 U.S.C. §112, second paragraph, and accordingly request withdrawal of the rejection.

REJECTION UNDER 35 U.S.C §101

Claim 46 was rejected under 35 U.S.C. §101 because the claimed recitation of a use does not set forth the steps involved in the process. Applicants respectfully note that original claim 46 was directed to a composition for pharmaceutical use, but for clarity the claim has been amended to explicitly state that the claim recites a "pharmaceutical composition". Applicants respectfully submit that withdrawal of this rejection is proper.

REJECTION UNDER 35 U.S.C §102

Claims 1-6, 17, 19, 22, 24 and 41-47 stand rejected under 35 U.S.C. §102(e) as being anticipated by **Pero et al.**, US Patent No. 6,538,038 (referred to hereafter as "**Pero**").

Applicants traverse. Applicants respectfully submit that the Publication is not prior art under 35 U.S.C. §102(e), since the claimed invention was invented by Applicants prior to the effective date of the reference. Submitted with this response is a Declaration under 37 C.F.R. §1.131 executed by Kevin G. Pinney, who is listed as an inventor in the subject application (the "Declaration"). Dr. Pinney is a research professor in synthetic organic chemistry at Baylor University, the Assignee of the subject application. As principal investigator and thesis advisor, Dr. Pinney supervised the work of several graduate and undergraduate students in his laboratory, many of whom worked on projects related to the reduction to practice of the present invention. The Declaration demonstrates his sole invention of the subject matter of Claims 1-6, 17, 19, 22, 24, and 41-47, prior to the effective date of **Pero**. The prior of invention of Compounds XVII and XVIII (Claims 17 and 19); generic trimethoxyphenyl-substituted indole ligands and their methods of use (Claims 1-3, 41-47); and positional isomers of these compounds (Claims 4-6, 22, and 24), are addressed separately as set forth below.

a) Prior Invention of Claims 17 and 19

Declarant Pinney unequivocally states that he conceived of Compound XVII and Compound XVIII (collectively, the "Compounds") as claimed in pending Claims 17 and 19 respectively, at a date prior to February 16, 2000, the effective date of **Pero**. The Declarant provides evidentiary support for this statement in the form of Exhibit 3 of the Declaration. Exhibit 3 is a copy of select pages from the May 1999 thesis manuscript of Feng Wang, a graduate student performing research in the laboratory of Kevin Pinney ("the Wang thesis"). The Wang thesis reports the synthesis of Compound 33, a non-hydroxylated analog of Compound XVII. Based on the promising anti-tubulin polymerization and anti-mitotic activity of Compound 33, Declarant Pinney proposed the synthesis of hydroxylated analogs of Compound 33. Several of these hydroxylated analogs are depicted in the Wang thesis, including Compound XVII. Declarant Pinney theorized that Compound XVII would have beneficial properties, namely anti-tubulin polymerization and anti-mitotic activity. Therefore, the Wang thesis provides *prima facie* evidence that Declarant Pinney conceived of Compound XVII prior to the effective filing date of **Pero**.

Submitted with this response is a priority claim under 35 U.S.C. §119(e) to Provisional Patent Application No. 60/154,639 which the Applicants filed in the USPTO on September 17,

1999 (hereinafter the "Provisional"). The Provisional provided a detailed description of the synthesis of Compound 33 (see Schemes 1-4, pages 11-15 and Example 1, pages 15-17), its tubulin binding activity (see Example 2, page 17), and its cytotoxicity towards tumor cells (see Examples 3 and 4, page 18). The Provisional also clearly indicated that a hydroxylated version of Compound 33 was envisioned (see page 19 of the Provisional, lines 20-24):

> "Phenolic groups may also have activity on these described indole ligands. The synthesis of any of these modified indole ligands will be very straight-forward for anyone skilled in the art, and often will only involve a different choice of initial starting materials. To prepare these alternative ligands, the same synthetic Schemes 1-4, or similar schemes with only slight modifications may be employed."

Having conceived of Compound XVII, Declarant Pinney recognized the importance of using phosphate ester derivatives of it and other hydroxylated indole compounds, since the addition of such a group would be expected to impart improved water solubility to the compound. Additionally, it was theorized that these phosphate prodrugs would be selectively dephosphorylated in the body to generate their active, hydroxylated counterparts. Most importantly, Declarant Pinney realized at the time of his invention that a phosphate ester group could also be necessary for improved tumor growth control through destruction of tumor vasculature. Applicants again stated in the Provisional that the phosphate ester portion is an important molecular feature for targeting the compound to sites of enhanced vascularization such as those found in a tumor (see page 5, lines 5-10). Having recognized the value of an indole phosphate ester prodrug, Declarant Pinney conceived and proposed the synthesis of Compound XVIII, the phosphate ester prodrug of Compound XVII, again, prior to the effective filing date of **Pero**.

After conceiving of the Compounds, Declarant Pinney then diligently proceeded to reduce his invention to practice. Discussion notes submitted as Exhibit 4 of the Declaration demonstrate that Dr. Pinney had initiated attempts to synthesize the Compounds as early as January 17, 2000, almost a full month before the filing date of **Pero**. Declarant Pinney devised a complete synthetic scheme for the Compounds (see "Scheme III" of Exhibit 5), based on methods well known in the art, and presented this scheme in a supplemental grant proposal to OXiGENE, the Assignee of the **Pero** application, prior to the filing date of **Pero**. This scheme appears in the present application in FIGs 12, 13 and 14.

Throughout the period from just prior to the February 16, 2000 filing date of **Pero** and up until at least May 30, 2000, Declarant Pinney enlisted technical assistance from an undergraduate chemistry student, Heather O'Dell, in order to prepare the Compounds. As

evidenced by several laboratory notebook entries from this period (see Exhibit 6 of Declaration), Ms. O'Dell made several attempts to obtain the necessary starting materials for the synthesis of the Compounds using the scheme devised by Dr. Pinney. Unfortunately, these attempts did not result in sufficient amounts of material, nor could it be conclusively demonstrated that these materials had indeed been successfully synthesized. Therefore, in order to expedite the synthesis of the materials, Declarant Pinney enlisted more experienced student and technician chemists in order to work on the reduction to practice of the Compounds. One of these students, Mallinath Hadimani, began to make progress towards this goal shortly after taking up work on the project as early as June 18th, 2000. In several laboratory notebook entries submitted as Exhibit 7 of the Declaration, Hadimani records progress towards the synthesis of Intermediate #1, the TBS-protected 2-bromo-3-hydroxy-4-methoxyacetophenone. Hadimani successfully obtained Intermediate #1 on July 23, 2000.

Hadimani continued with diligence in his laboratory work through and after September 15, 2000, at which time Applicants constructively reduced their invention to practice by filing a PCT Application designating the United States of America (International PCT Application No. PCT/US00/25408) and listing the same description and claims as currently pending in the subject Application.

Declarant Pinney continued with diligence to actually reduce the invention to practice by synthesizing the Compounds. Declarant Pinney assembled a larger team of chemists (see Exhibit 8 of Declaration), all of whom independently but under Dr. Pinney's direction worked towards this goal. Notebook entries from one of these chemists, Jimmy Kessler, demonstrate that he successfully obtained Intermediate #2 on September 28, 2000, and Compound XVII shortly thereafter, on October 10, 2000 (see Exhibit 9 of Declaration). Mallinath Hadimani continued on with the phosphorylation of Compound XVII, and successfully obtained Compound XVIII on November 13, 2000, as evidenced by his notebook entries in Exhibit 10.

When taken together, all of the foregoing activities demonstrate reasonable and continuous diligence by Dr. Pinney in both constructively and actually reducing his invention to practice.

b) Prior Invention of Claims 1-6, 22, 24, 41-47

In the view of the Examiner, Claims 1-6, 22, and 24 are also anticipated by Pero. Applicants traverse this rejection for at least one of the following reasons. Firstly, in order to anticipate Claims 4-6, 22, and 24, **Pero** must teach each and every element and feature of these claims. The only compounds discussed or described in the Pero application that are relevant to these claims are Compounds XVII and XVIII. But as noted above, the **Pero** reference has been

removed as prior art since these compounds were invented prior to the effective **Pero** reference date. Accordingly, Applicants submit that the 102(e) rejection does not apply to Claims 4-6, 22, and 24 as amended, and should be withdrawn.

Secondly, as the Declarants note, the subject matter of Claims 1-6 was clearly set out in claims accompanying the Provisional. For example, Provisional claim 5 is a substantial duplicate of pending Claim 1 in the Application as it recites a generic structure for indole-based anti-mitotic agents containing a "phenolic moiety", *i.e.,* an aryl ring with a hydroxyl group. Therefore the subject matter of these claims was constructively reduced to practice at least as early as the priority patent application. Thirdly, the Declaration submitted herewith establishes prior invention of the subject matter of Claims 17 and 19, and therefore also the subject matter of Claims 1-3, since Claim 1 as a whole reads on the species of Claim 17 and Claim 1 and 2 both read on the species claimed in Claim 19 (see MPEP 715.02).

Rejected claims 41-47 are thus not anticipated by **Pero** for at least the same reasons as recited with regard to the independent claims from which they depend.

CONCLUSION

In view of the aforementioned remarks and amendments, the Applicants believe that each of pending claims is in condition for allowance. Reconsideration, withdrawal of the rejections, and passage of the case to issue is respectfully requested. A notice to this effect is earnestly solicited.

If, upon receipt and review of this amendment, the Examiner believes that the present application is not in condition for allowance and that changes can be suggested which would place the claims in allowable form, the Examiner is respectfully requested to call Applicant's undersigned counsel at the number provided below.

Respectfully submitted,

Ivor R. Elrifi, Reg. No. 39,529
Naomi S. Biswas, Reg. No. 38,384
Nicholas P. Triano III, Reg. No. 36,397
Attorneys for Applicants
MINTZ LEVIN COHN FERRIS
GLOVSKY & POPEO PC
Customer Number 30623
Tel: (617) 542-6000
Fax: (617) 542-2241

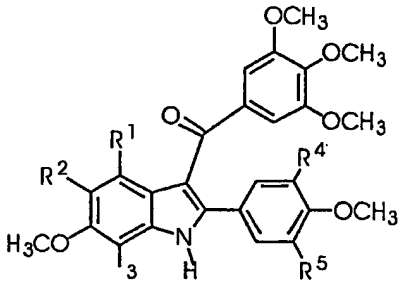
Dated: March 9, 2004

Exhibit 1 – Pending claims

(see attached)

What is claimed is:

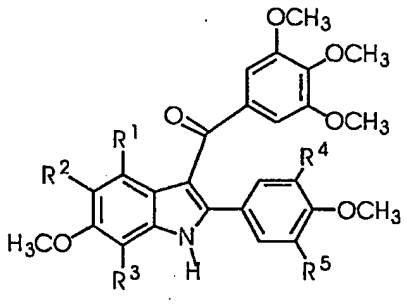
1. A compound of the structure:



wherein

R^1 through R^5 contain at least one phenolic moiety or at least one amine group (NH_2, NHR^1, or NR^6R^7 where R^6 and R^7 are the same or different alkyl having up to 8 carbon atoms), benzyl, or aryl while the remaining R^1 through R^5 are hydrogen.

2. A compound of the structure:

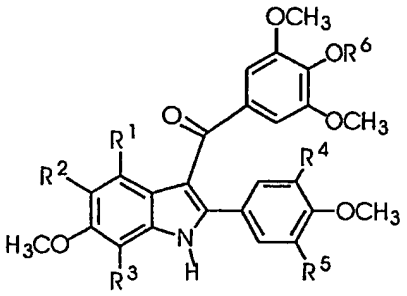


wherein

R^1 through R^5 contain at least one phosphate ester moiety ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where

R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different, benzyl, or aryl while the remaining R^1 through R^5 are hydrogen.

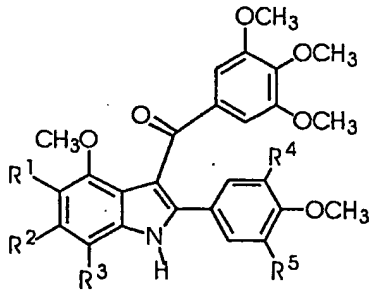
3. A compound of the structure:



wherein

R^1 through R^5 contain at least one phosphate ester moiety ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), benzyl, or aryl while the remaining R^1 through R^5 are hydrogen, and R^6 is hydrogen or alkyl.

4. A compound of the structure:

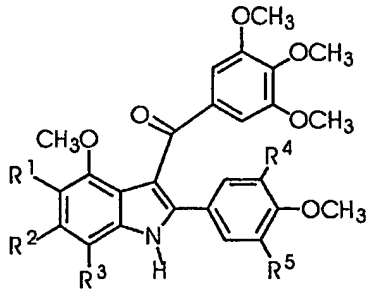


wherein

R^1 through R^5 contain at least one phenolic moiety or at least one amine (NH_2, NHR^1, or NR^6R^7 where R^6 and R^7 the same or different alkyl having up to 8

carbon atoms, benzyl, or aryl groups) while the remaining R^1 through R^5 are a hydrogen.

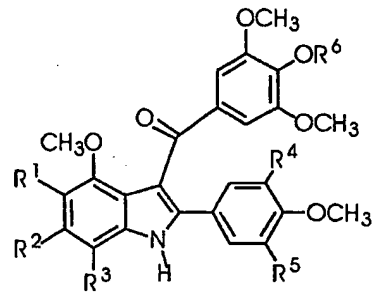
5. A compound of the structure:



wherein

R^1 through R^5 contain at least one phosphate ester moiety ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), benzyl, or aryl while the remaining R^1 through R^5 are hydrogen.

6. A compound of the structure:

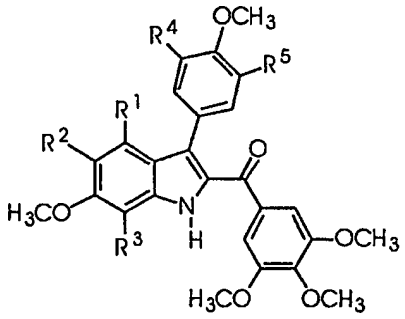


wherein

R^1 through R^5 contain at least one phosphate ester moiety ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M = a cation or ($-NP(O)(OR)_2$) where

R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), or benzyl, or aryl groups, while the remaining R^1 through R^5 are a hydrogen and R^6 is hydrogen or alkyl.

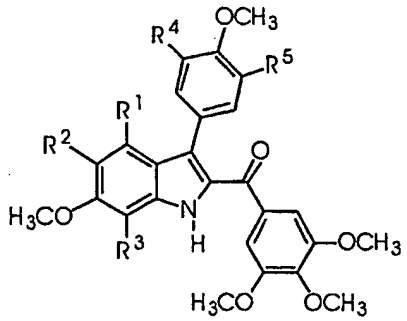
7. A compound of the structure:



wherein

R^1 through R^5 contain at least one phenolic moiety or at least one amine group (NH_2, NHRor NR^6R^7 where R^6 and R^7 are the same or different alkyl having up to 8 carbon atoms may be the same or different), or benzyl, or aryl groups) while the remaining R^1 through R^5 are a hydrogen.

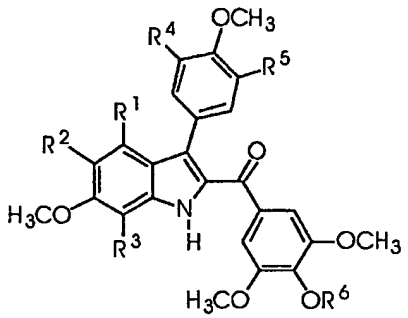
8. A compound of the structure:



wherein

R^1 through R^5 contain at least one phosphate ester ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), benzyl, or aryl while the remaining R^1 through R^5 are hydrogen.

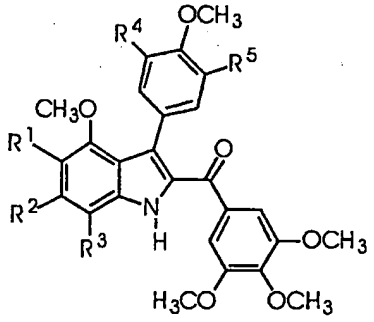
9. A compound of the structure:



wherein

R^1 through R^5 contain at least one phosphate ester ($-OP(O)(O^-M^+)_2$) or phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), benzyl, or aryl, while the remaining R^1 through R^5 are hydrogen, and R^6 is hydrogen or alkyl.

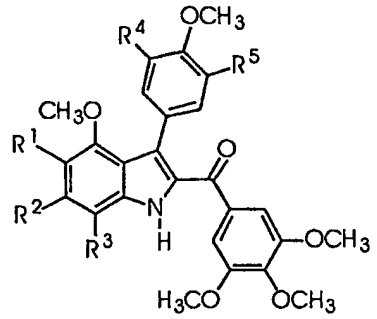
10. A compound of the structure:



wherein

R^1 through R^5 contain at least one phenolic moiety or at least one amine group (NH_2, NHR^1, or NR^6R^7 where R^6 and R^7 are the same or different alkyl having upt to 8 carbon atoms, benzyl, or aryl) while the remaining R^1 through R^5 are a hydrogen.

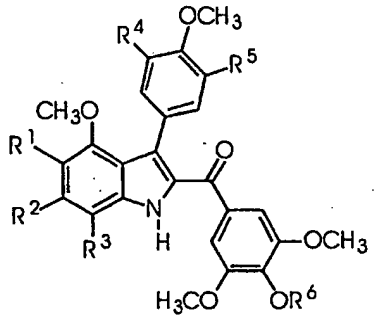
11. A compound of the structure:



wherein

R^1 through R^5 contain at least one phosphate ester ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), benzyl, or aryl, while the remaining R^1 through R^5 are hydrogen.

12. A compound of the structure:

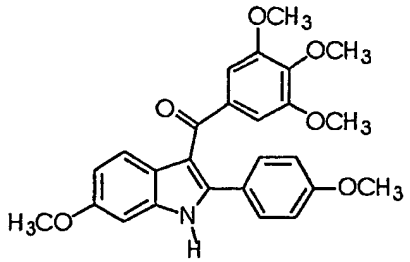


wherein

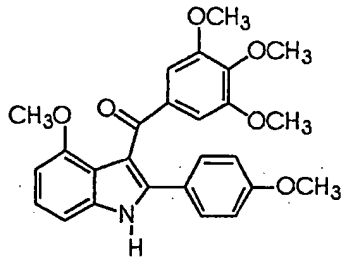
R^1 through R^5 contain at least one phosphate ester moiety ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an

alkyl with up to 8 carbon atoms (the two R groups are the same or different), benzyl, or aryl while the remaining R^1 through R^5 are hydrogen, and R^6 is hydrogen or alkyl.

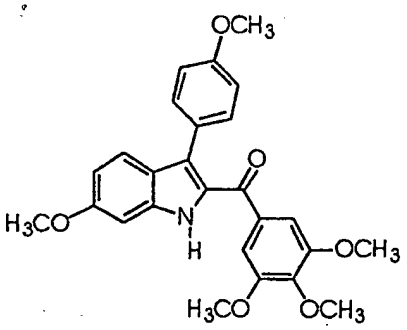
13. A compound of the structure:



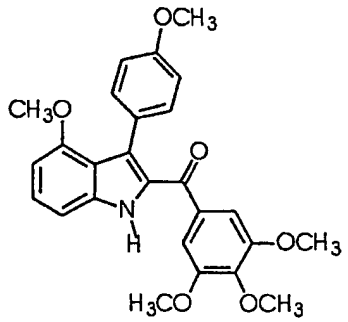
14. A compound of the structure:



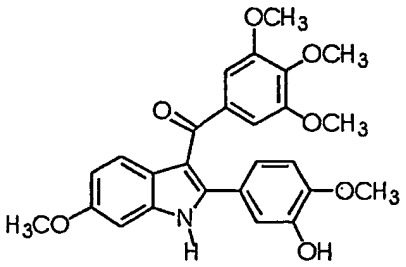
15. A compound of the structure:



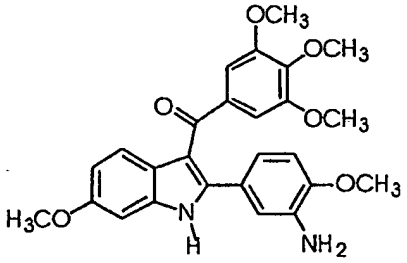
16. A compound of the structure:



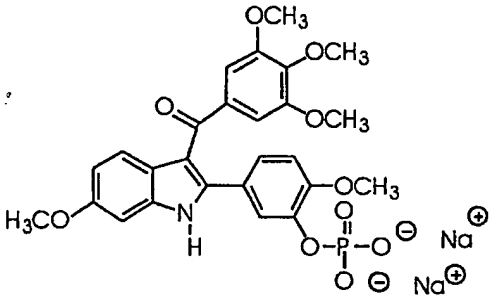
17. A compound of the structure:



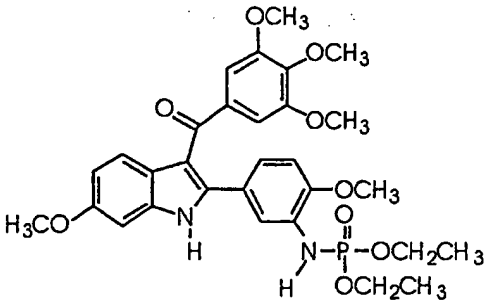
18. A compound of the structure:



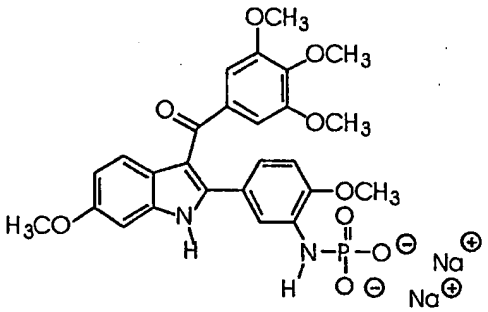
19. A compound of the structure:



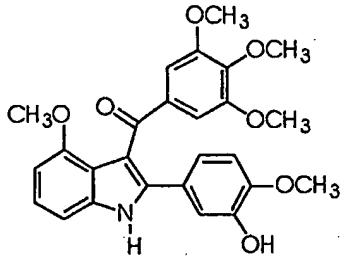
20. A compound of the structure:



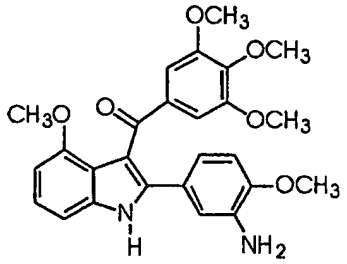
21. A compound of the structure:



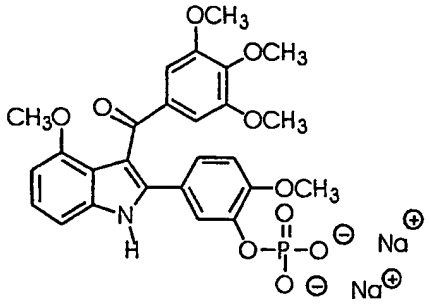
22. A compound of the structure:



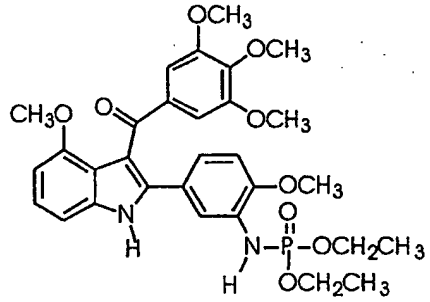
23. A compound of the structure:



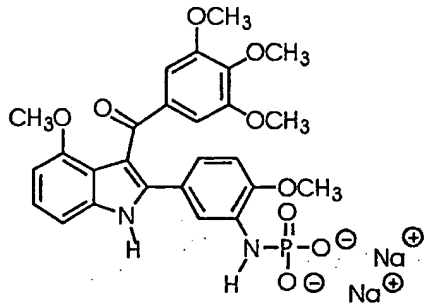
24. A compound of the structure:



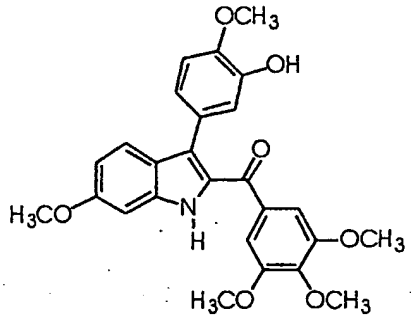
25. A compound of the structure:



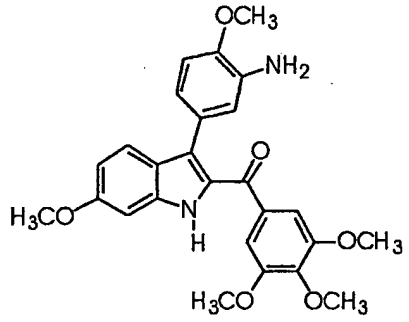
26. A compound of the structure:



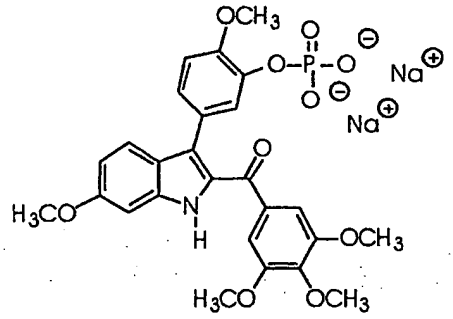
27. A compound of the structure:



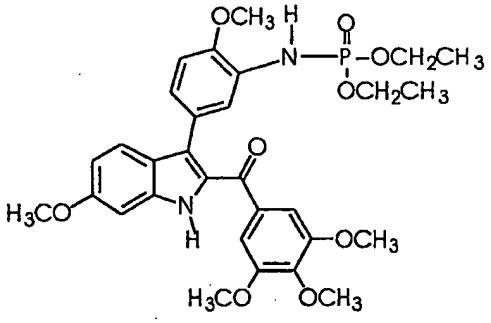
28. A compound of the structure:



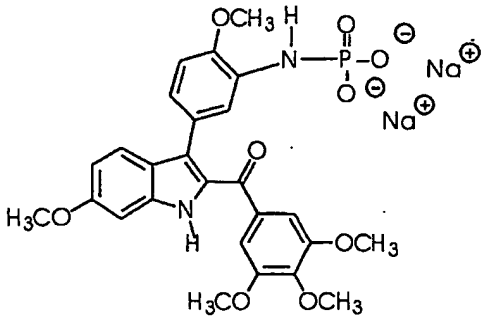
29. A compound of the structure:



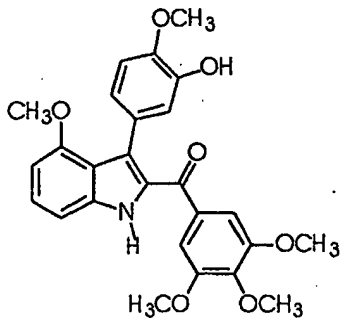
30. A compound of the structure:



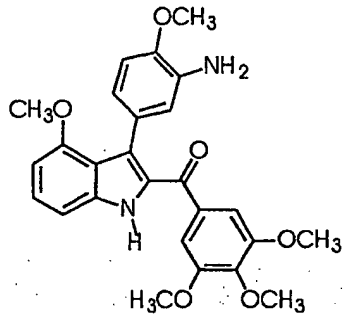
31. A compound of the structure:



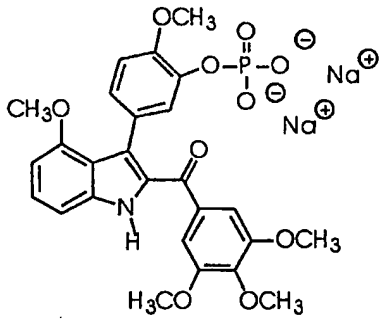
32. A compound of the structure:



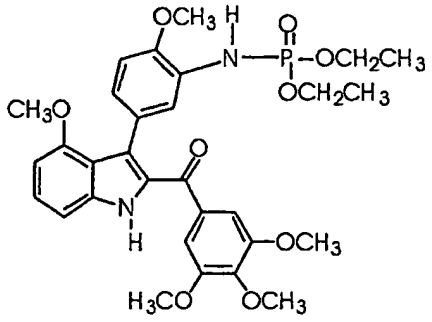
33. A compound of the structure:



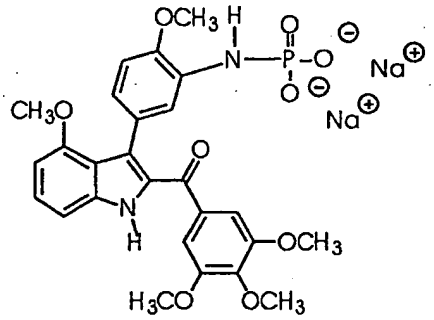
34. A compound of the structure:



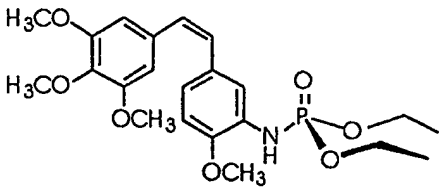
35. A compound of the structure:



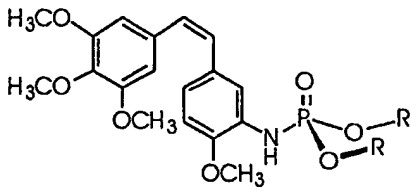
36. A compound of the structure:



37. A compound of the structure:



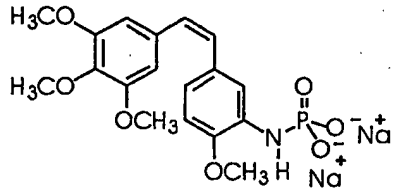
38. A compound of the structure:



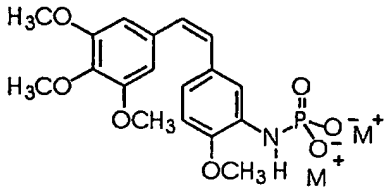
wherein

R is chosen to be any appropriate alkyl or branched alkyl having up to 8 carbon atoms, the two R groups may be the same or different.

39. A compound of the structure:



40. A compound of the structure:



wherein

M^+ is a cation.

41. A method for inhibitiing tubulin polymerization by contacting a tubulin-containing system with an effective amount of a compound described in any of claims 1-40.

42. The method of claim 41 wherein said system is in a tumor cell.

43. A method of treating a host afflicted with a neoplastic disease by administering to said host a compound described in any of claims 1-40.

44. The method of claims 41, wherein the contacted system is located in a patient.

45. The method of claim 41 described further as for treating cancer, wherein said cancer may be chosen from the group containing leukemia, lung, colon, thyroid, CNS, melanoma, ovarian, renal, prostate, and breast cancers.

46. A preparation for pharmaceutical use containing a compound from any of claims 1-40 as an active component along with a pharmaceutically acceptable carrier.

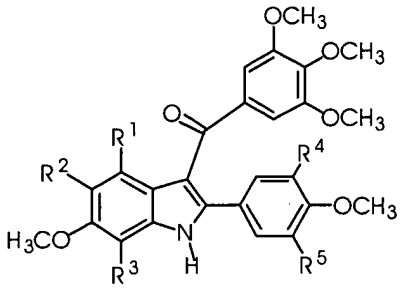
47. A method for selectively targeting and destroying tumor vasculature comprising administering an effective amount of a compound described in any of claims 1-40.

Applicant(s): Kevin G. Pinney
Appl'n No. 10/070,484

Exhibit 2 – Amended claims in Response to Office Action accompanying this petition

(see attached)

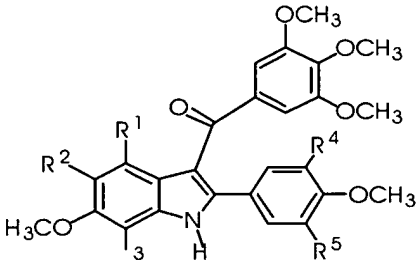
1. (Currently Amended) A compound of the structure:



wherein

R^1 through R^5 contain at least one hydroxyl group ~~phenolic moiety~~ or at least one amine group (NH_2, ~~NHR^1~~NHR^6, or NR^6R^7 where R^6 and R^7 are the same or different alkyl having up to 8 carbon atoms), ~~benzyl, or aryl~~ while the remaining R^1 through R^5 are hydrogen.

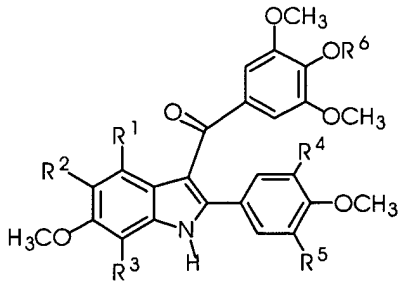
2. (Currently Amended) A compound of the structure:



wherein

R^1 through R^5 contain at least one phosphate ester ~~moiety~~ ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), ~~benzyl, or aryl~~ while the remaining R^1 through R^5 are hydrogen.

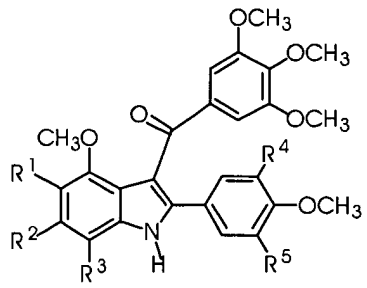
3. (Currently Amended) A compound of the structure:



wherein

R^1 through R^5 contain at least one phosphate ester ~~moiety~~ ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), ~~benzyl, or aryl~~ while the remaining R^1 through R^5 are hydrogen, and R^6 is hydrogen or alkyl.

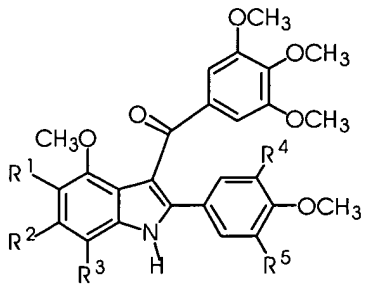
4. (Currently Amended) A compound of the structure:



wherein

R^1 through R^5 contain at least one hydroxyl ~~phenolic moiety~~ or at least one amine (NH_2, ~~NHR~~ NHR^6, or NR^6R^7 where R^6 and R^7 the same or different alkyl having up to 8 carbon atoms~~, benzyl, or aryl groups~~) while the remaining R^1 through R^5 are a hydrogen.

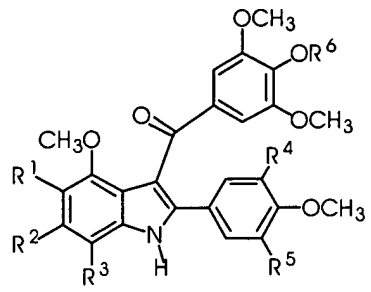
5. (Currently Amended) A compound of the structure:



wherein

R^1 through R^5 contain at least one phosphate ester ~~moiety~~ ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), ~~benzyl, or aryl~~ while the remaining R^1 through R^5 are hydrogen.

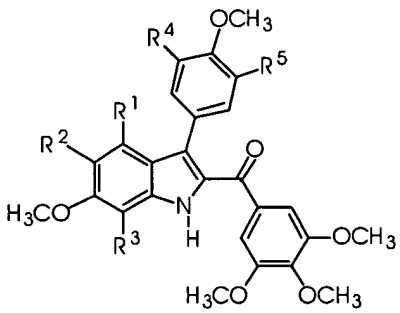
6. (Currently Amended) A compound of the structure:



wherein

R^1 through R^5 contain at least one phosphate ester ~~moiety~~ ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is= a cation or ($-NP(O)(OR)_2$) where R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), ~~or benzyl, or aryl groups,~~ while the remaining R^1 through R^5 are a hydrogen and R^6 is hydrogen or alkyl .

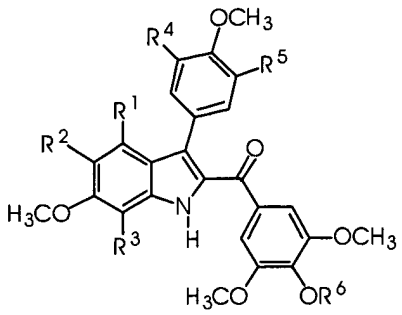
7. (Currently Amended) A compound of the structure:



wherein

R^1 through R^5 contain at least one hydroxyl group ~~phenolic moiety~~ or at least one amine group (NH_2, ~~NHR~~NHR^6 or NR^6R^7 where R^6 and R^7 are the same or different alkyl having up to 8 carbon atoms ~~may be the same or different), or benzyl, or aryl groups~~) while the remaining R^1 through R^5 are a hydrogen.

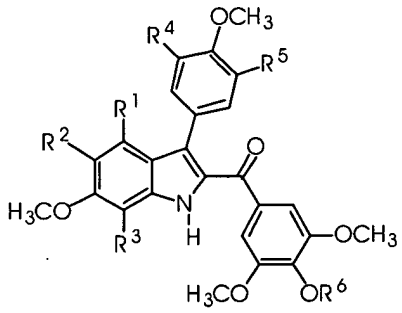
8. (Currently Amended) A compound of the structure:



wherein

R^1 through R^5 contain at least one phosphate ester ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), ~~benzyl, or aryl~~ while the remaining R^1 through R^5 are hydrogen.

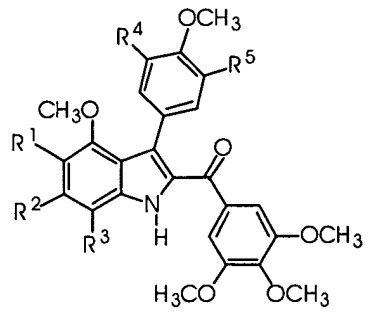
9. (Currently Amended) A compound of the structure:



wherein

R^1 through R^5 contain at least one phosphate ester ($-OP(O)(O^-M^+)_2$) or phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), ~~benzyl, or aryl,~~ while the remaining R^1 through R^5 are hydrogen, and R^6 is hydrogen or alkyl.

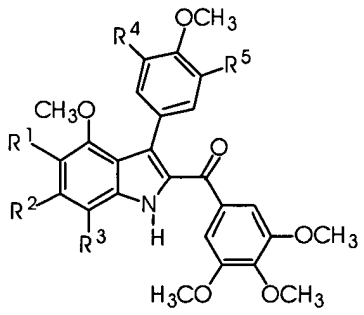
10. (Currently Amended) A compound of the structure:



wherein

R^1 through R^5 contain at least one hydroxyl group ~~phenolic moiety~~ or at least one amine group (NH_2, ~~NHR~~NHR^6, or NR^6R^7 where R^6 and R^7 are the same or different alkyl having upt to 8 carbon atoms, benzyl, or aryl) while the remaining R^1 through R^5 are a hydrogen.

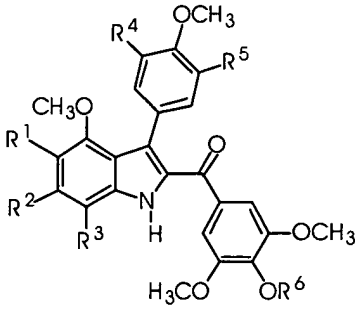
11. (Currently Amended) A compound of the structure:



wherein

R^1 through R^5 contain at least one phosphate ester ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), ~~benzyl, or aryl,~~ while the remaining R^1 through R^5 are hydrogen.

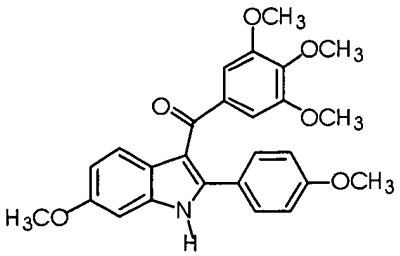
12. (Currently Amended) A compound of the structure:



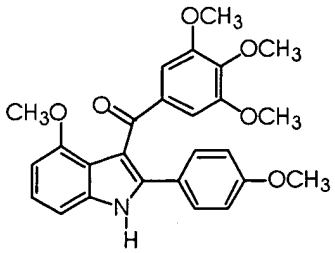
wherein

R^1 through R^5 contain at least one phosphate ester ~~moiety~~ ($-OP(O)(O^-M^+)_2$) or a phosphoramidate ($-NP(O)(O^-M^+)_2$) where M is a cation or ($-NP(O)(OR)_2$) where R is an alkyl with up to 8 carbon atoms (the two R groups are the same or different), ~~benzyl, or aryl~~ while the remaining R^1 through R^5 are hydrogen, and R^6 is hydrogen or alkyl.

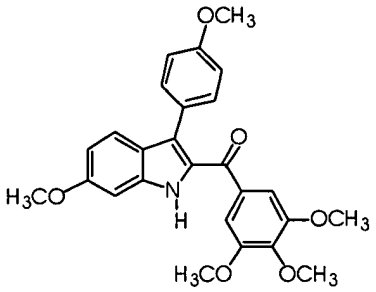
13. (Original) A compound of the structure:



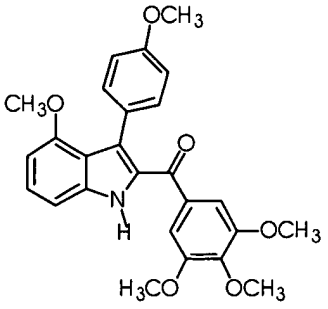
14. (Original) A compound of the structure:



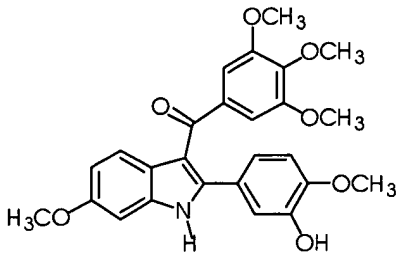
15. (Original) A compound of the structure:



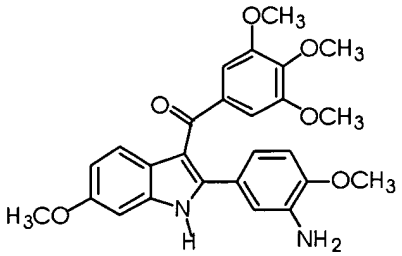
16. (Original) A compound of the structure:



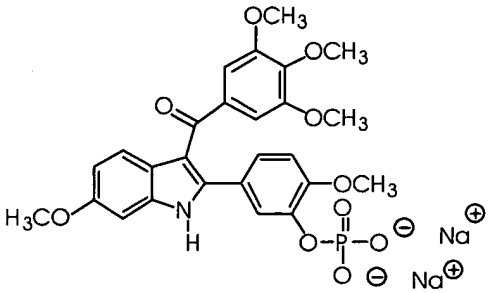
17. (Original) A compound of the structure:



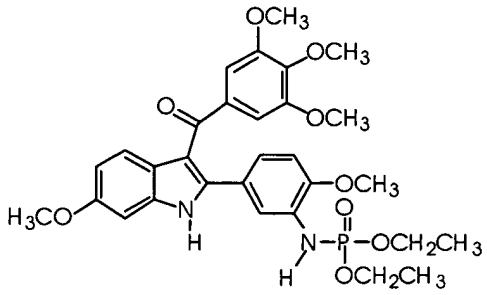
18. (Original) A compound of the structure:



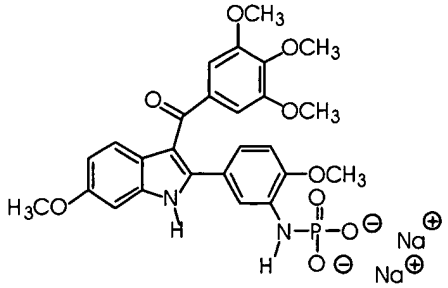
19. (Original) A compound of the structure:



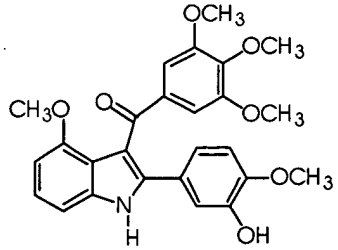
20. (Original) A compound of the structure:



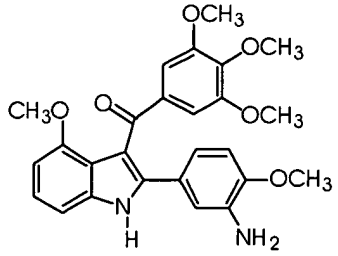
21. (Original) A compound of the structure:



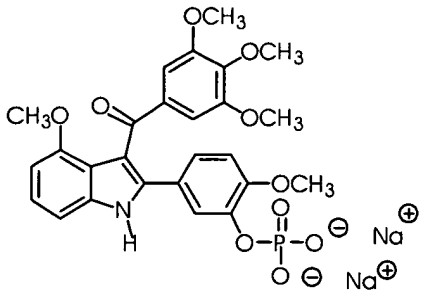
22. (Original) A compound of the structure:



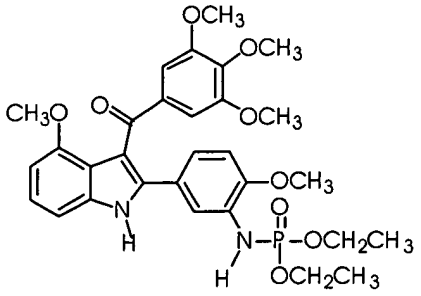
23. (Original) A compound of the structure:



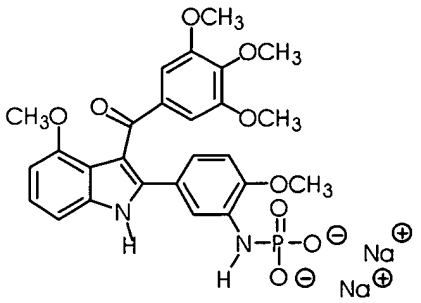
24. (Original) A compound of the structure:



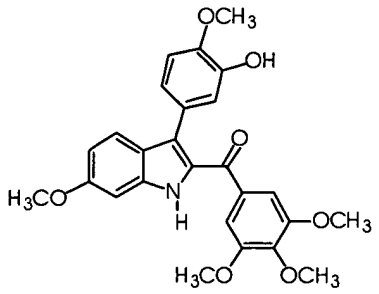
25. (Original) A compound of the structure:



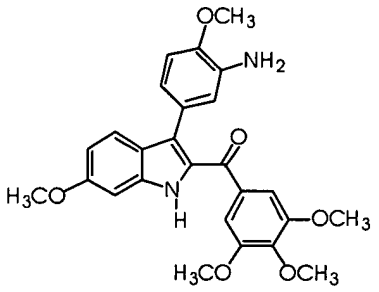
26. (Original) A compound of the structure:



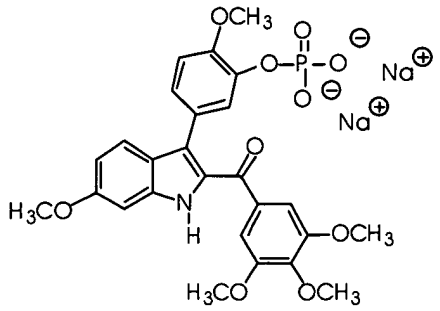
27. (Original) A compound of the structure:



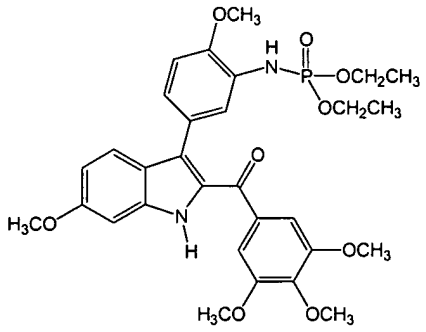
28. (Original) A compound of the structure:



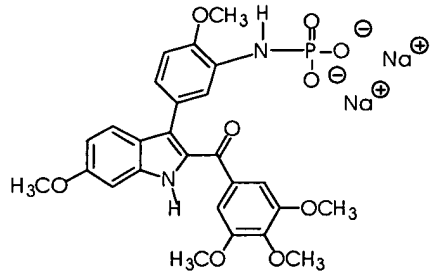
29. (Original) A compound of the structure:



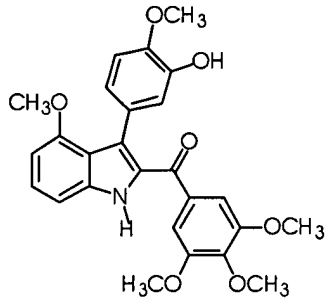
30. (Original) A compound of the structure:



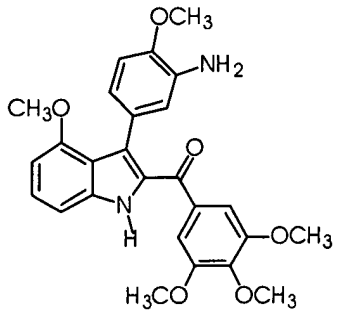
31. (Original) A compound of the structure:



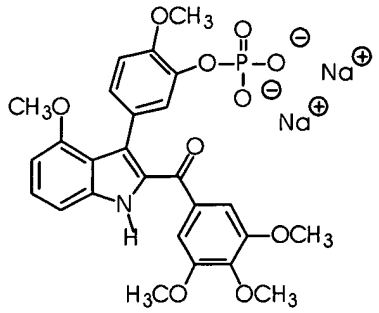
32. (Original) A compound of the structure:



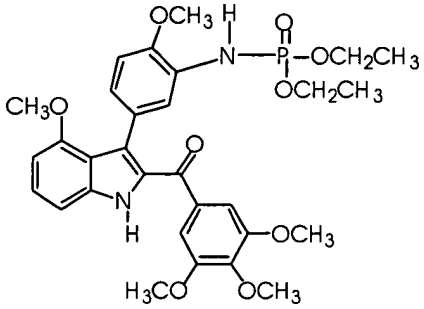
33. (Original) A compound of the structure:



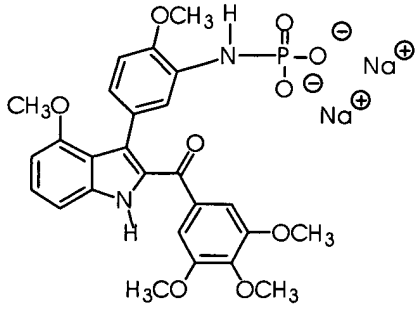
34. (Original) A compound of the structure:



35. (Original) A compound of the structure:



36. (Original) A compound of the structure:



37. (Cancelled)

38. (Cancelled)

39. (Cancelled)

40. (Cancelled)

41. (Currently Amended) A method for inhibiting tubulin polymerization by contacting a cell ~~tubulin-containing system~~ with an effective amount of a compound described in any one of claims 1-36~~40~~.

42. (Currently amended) The method of claim 41 wherein said cell ~~system~~ is ~~in~~ a tumor cell.

43. (Currently Amended) A method of treating a mammal ~~host~~ afflicted with a neoplastic disease by administering to said mammal ~~host~~ a therapeutically effective amount of a compound described in any one of claims 1-36~~40~~.

44. (Currently Amended) The method of claims 41, wherein the contacted cell ~~system~~ is located in a patient.

45. (Currently Amended) ~~The method of claim 41 described further as for~~ A method for treating cancer by administering to a patient in need thereof, a therapeutically effective amount of a compound described in any one of claims 1-36, wherein said cancer is selected from the group consisting of ~~may be chosen from the group containing~~ leukemia, lung cancer, colon cancer, thyroid cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, pancreatic cancer, and breast cancers.

46. (Currently Amended) A ~~preparation for pharmaceutical use containing~~ pharmaceutical composition comprising a compound from any one of claims 1-36~~40~~ as an active component along with a pharmaceutically acceptable carrier.

47. (Currently Amended) A method for selectively ~~targeting and~~ destroying tumor vasculature in a patient comprising administering an effective amount of a compound described in any one of claims 1-36~~40~~.

Exhibit 3

(see attached)

Stereoselective Synthesis of Conjugated Diene Systems and Design of New Tubulin Polymerization Inhibitors as Antimitotic Agents

A Thesis Submitted to the Faculty of

Baylor University

in Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

by

Feng Wang

Waco, Texas

May 1999

ABSTRACT

Stereoselective Synthesis of Conjugated Diene Systems and Design of New Tubulin Polymerization Inhibitors as Antimitotic Agents

Feng Wang

Mentor: Kevin G. Pinney, Ph.D.

A new synthetic methodology has been developed for the synthesis of highly functionalized, conjugated dienes from alkynyl oxirane precursors by treatment with an appropriate organocuprate reagent. The reaction proceeds in a highly stereospecific fashion in each case. The stereochemistry of the methyl substituted diene (as a *bis* p-nitrobenzoate derivative) and the phenyl substituted diene was determined by x-ray crystallographic analysis.

An indole-based ligand has been prepared as a new antimitotic, anticancer agent based on previous work with benzo[*b*]thiophene systems. The indole target compound was prepared in good yield, and biological evaluation indicates that this ligand has remarkable activity.

The methyl substituted conjugated diene adopts an *S-cis* conformation which might be utilized as a peptidomimetic β-turn analog. Accordingly, we have modified this ligand to incorporate methoxyaryl motifs reminiscent of

colchicine and combretastatin A-4, both potent antimitotic, antitumor agents. Biological evaluation of these compounds is in progress.

CHAPTER FOUR

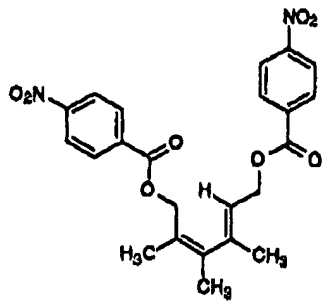
Conclusions

The first project has focused on the development of a new synthetic methodology for the stereoselective synthesis of highly functionalized, conjugated dienes from alkynyl oxirane precursors. The conjugated diene is obtained in one step from the requisite alkynyl oxirane by treatment with an appropriate organocuprate reagent. The scope of the reaction is such that it tolerates a wide variety of alkyl and aryl cuprate reagents. The reaction appears to proceed in a highly stereospecific fashion and tolerate a wide variety of functionality on both the alkynyl oxirane as well as within the organocuprate reagent itself. The allylic alcohol "handles" are convenient sites for the incorporation of this molecular fragment into more complex molecular systems. The stereochemistry of the methyl substituted diene was determined by x-ray crystallographic analysis of the *bis p*-nitrobenzoate derivative (structure determined through a collaborative effort with Professor William Watson, Texas Christian University) as well as the phenyl substituted conjugated diene (structure determined through a collaborative effort with Professor Donald Mullica, Baylor University). Two mechanisms have been proposed to explain the different stereochemistry observed in this study.

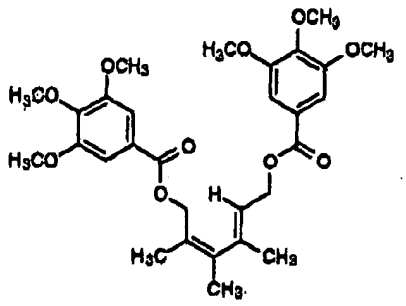
In the future, this new methodology will be applied toward the synthesis of more complex molecular systems. Since the *S-cis* conformation is obtained readily, these conjugated dienes may prove useful as diene partners in Diels-Alder reactions applied toward the preparation of various ring systems.

In addition, efforts will be directed toward selective functionalization of the diol in order to facilitate the incorporation of these conjugated dienes as building blocks for the synthesis of target compounds which contain this structural motif.

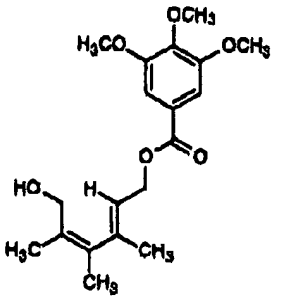
The second project has focused on the design and synthesis of an indole-based analog as a new antimitotic agent, as well as the preparation of conjugated diene-based compounds as β-turn peptidomimetic analogs. The target compounds **33, 25, 26, 27, 28** and **29** were achieved in good yield. Compounds **33, 21, 25, 26** and **27** (Figure 19) have been evaluated in terms of their cytotoxicity against selected human cancer cell lines (collaboration with Professor George Pettit, Arizona State University)[108] as well as their ability to inhibit tubulin polymerization (by Dr. Ernest Hamel, National Cancer Institute).[109] Compounds **25** and **27** have marginal activity, while compounds **26** and **21** have no activity against human cancer cell lines. Compounds **28** and **29** are currently being evaluated for their biological activity. The evaluation of the indole-based analog **33** shows that this ligand demonstrate remarkable cytotoxicity against selected human cancer cell lines (GI_{50}<1x10^-3 μg/mL) as well as excellent tubulin inhibition (IC_{50}=0.5-1.0 μM).



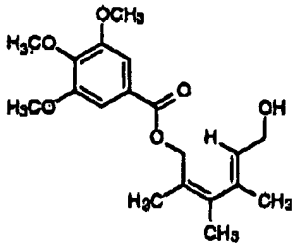
21



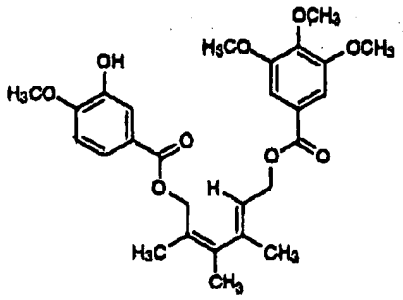
25



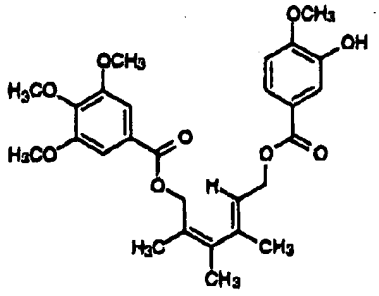
27



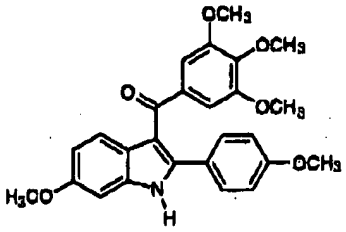
26



29



28



33

Figure 19. Compounds for Biological Evaluation

Based on these promising results, several new analogs of the indole 33 will be synthesized, and evaluated for their biological activity (Figure 20).

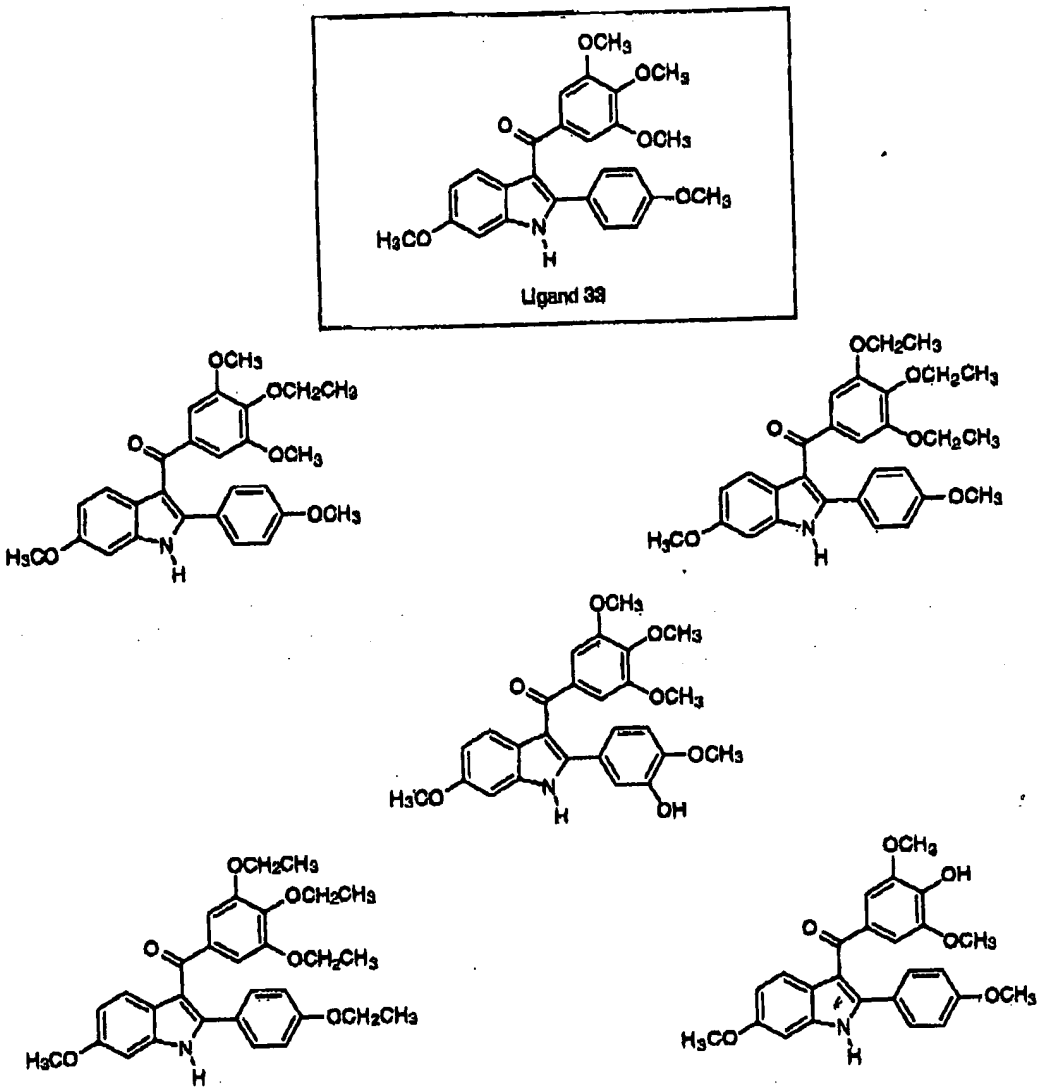
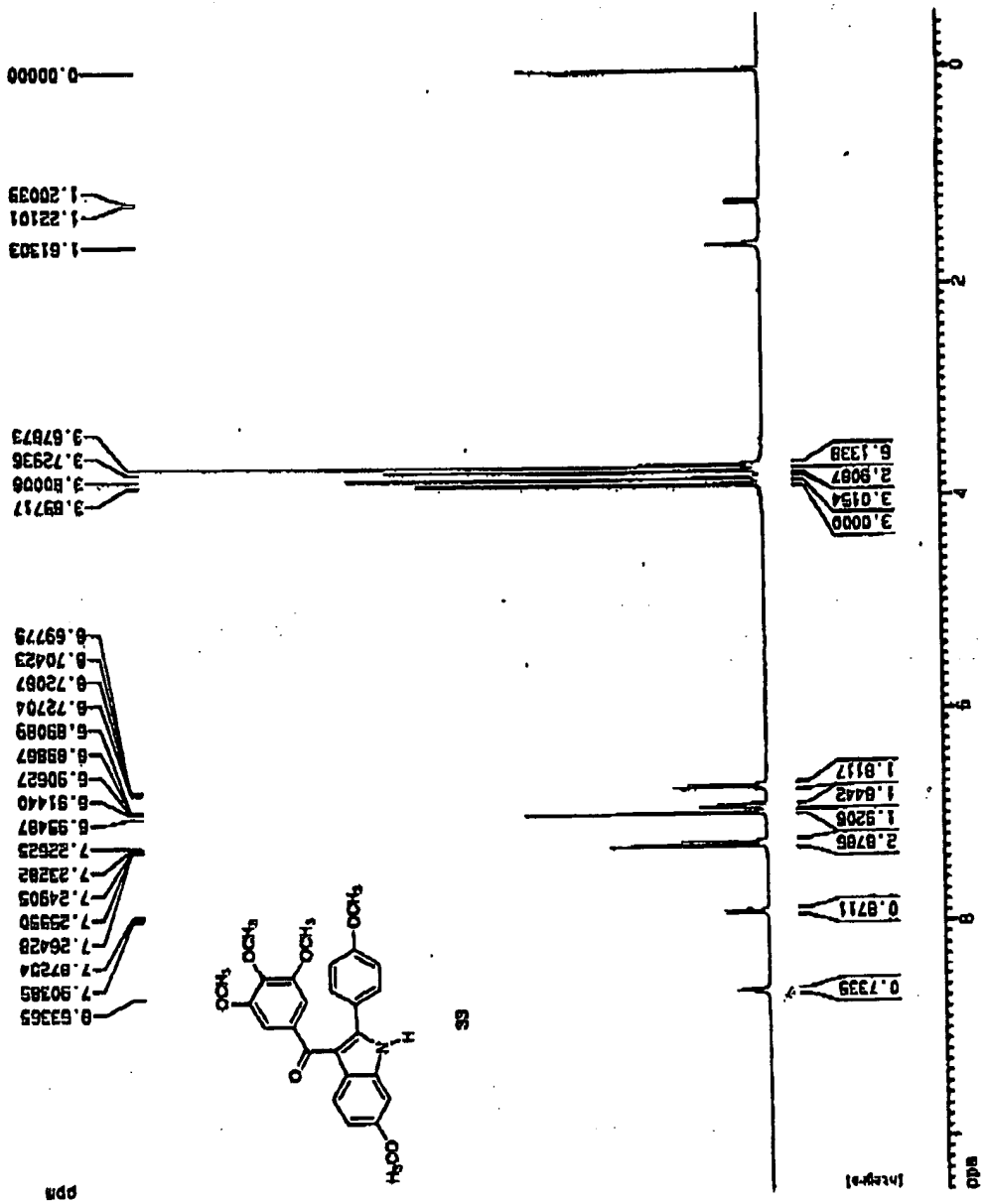


Figure 20. Future Ligands Targeted as Inhibitors of Tubulin Polymerization


0.00000
1.20039
1.22101
1.61303
3.67873
3.72936
3.80006
3.89717
8.69779
8.70423
8.72087
8.72704
8.89089
8.89867
8.90627
8.91440
8.93487
7.22625
7.23282
7.24905
7.25950
7.26428
7.87254
7.90385
8.53365
3.00000
3.01547
2.90877
6.1338
1.81127
1.8442
1.9208
2.8766
0.8711
0.7339
OCH3
OCH3
OCH3
OCH3
H
N
O
H3COOC
ppm

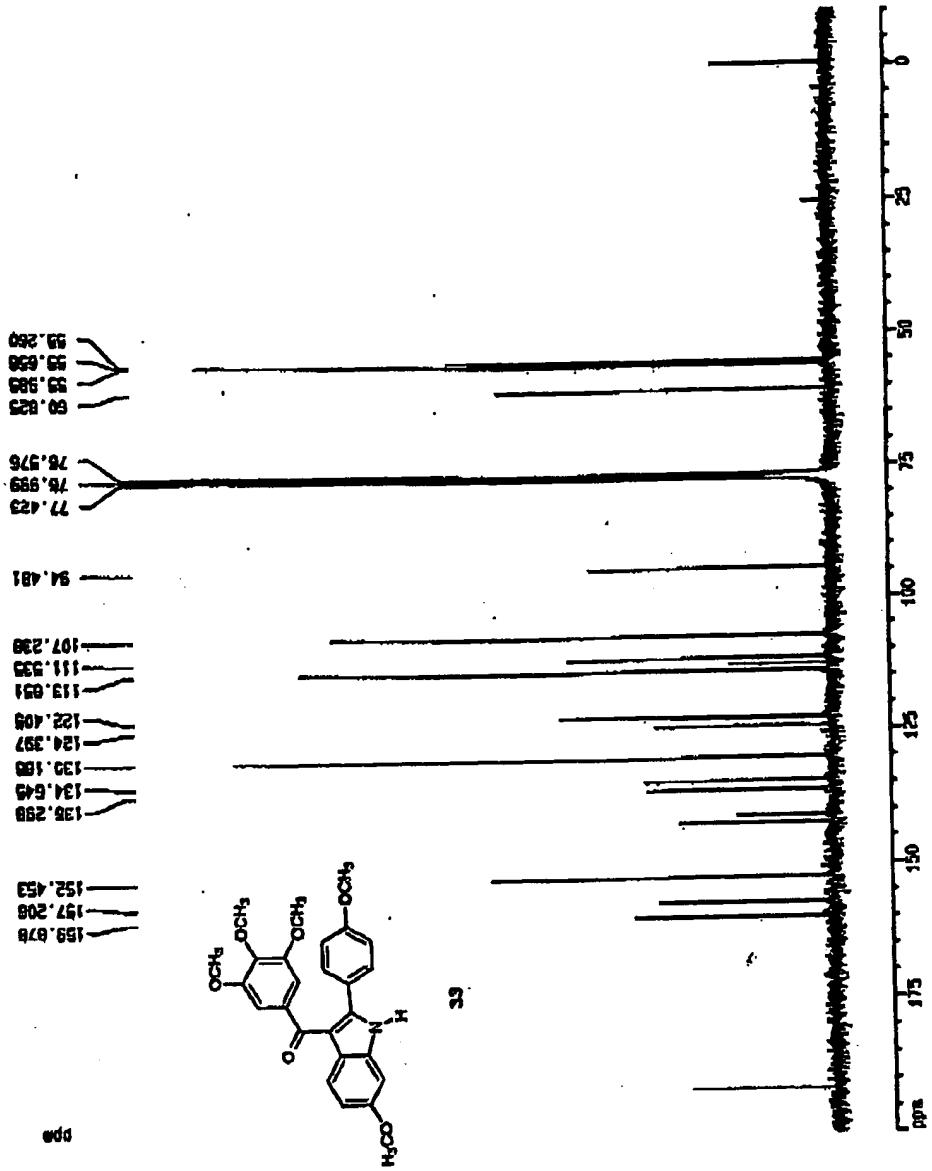
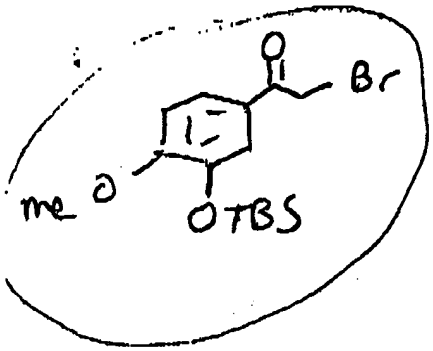
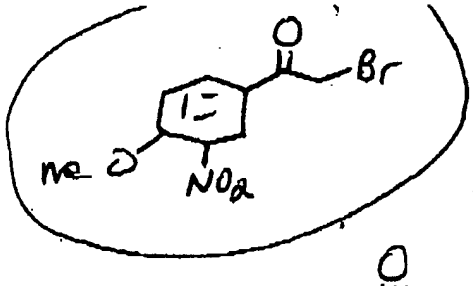
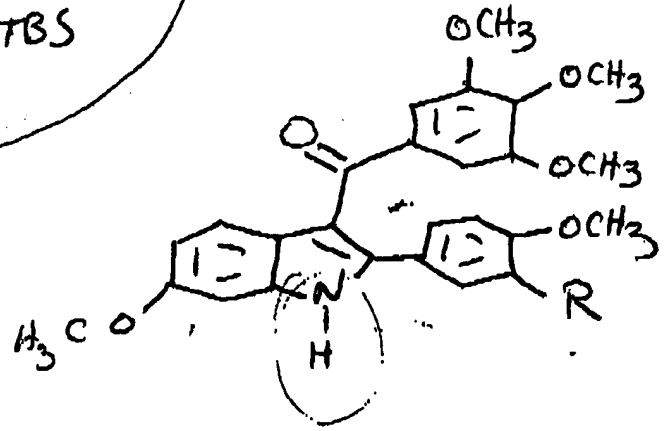

55.260
55.658
55.985
60.825
76.976
76.999
77.425
94.481
107.238
111.333
113.051
122.408
124.357
133.180
134.649
136.298
152.453
157.208
159.878
ppm
0
25
50
75
100
125
150
175
ppm
OCH3
OCH3
OCH3
OCH3
O
H
H3CO
33

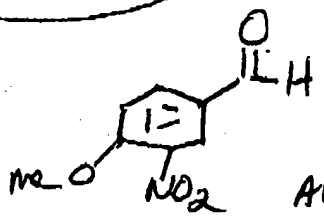
Exhibit 4

(see attached)









Aldrich

$R = OH$

$R = -O-\overset{O}{\overset{\|}{P}}(-O^{\ominus}Na^{\oplus})(O^{\ominus}Na^{\oplus})$

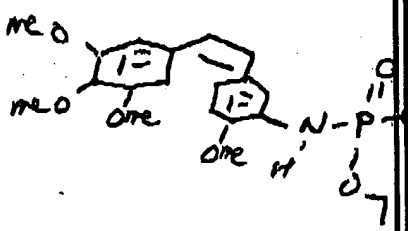
Heather O'Dell ✓

Johnny Nexstel ✓

Nandeo ✓

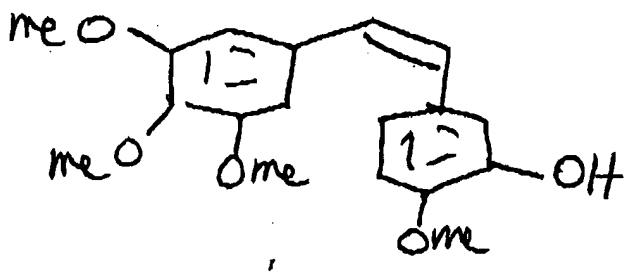
$R = -NH_2$

$R = -N(H)-\overset{O}{\overset{\|}{P}}(OEt)(OEt)$



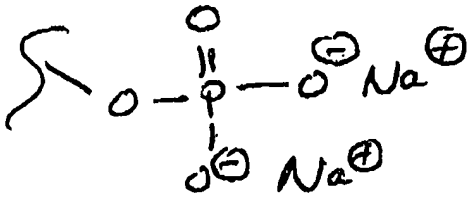
Zhi has made another one

Mallinckrodt



CA-4

Bob Pettit



CA-4P

Disodium Salt

increase solubility

Selectivity for tumor cells

CA-4P is now referred to as

a "~~CA~~ Tumor Vasculature Destruction Agent"

others

Antiangiogenesis

(1) Long standing interest in molecular ~~reco~~recognition for tubulin binding sites

- colchicine site

(2) Partnership with Oxigene

Exhibit 5

(see attached)

OXIGENE, INC. / BAYLOR UNIVERSITY

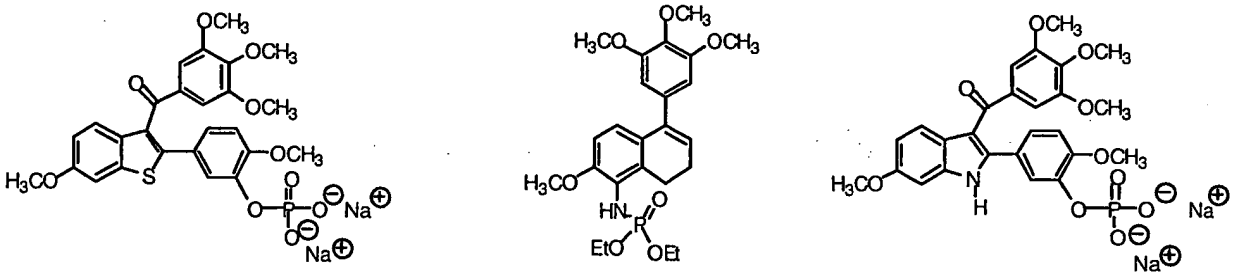
YEAR 1 (JUNE 1, 1999 - MAY 31, 2000) GRANT SUPPLEMENT

FUNDING REQUEST SUBMITTED: FEBRUARY 15, 2000

PROJECT 2 SUPPLEMENT: SYNTHESIS OF TUMOR VASCULATURE TARGETING AGENTS

Professor Kevin G. Pinney. Principal Investigator

Introduction: A number of phosphate prodrugs that we have prepared, to date, demonstrate excellent specificity in terms of targeting tumor vasculature. These compounds follow the now established protocol for tumor specific vascular targeting drugs; in other words, the phosphate prodrug form is not cytotoxic while the parent phenolic (or amino) precursor is cytotoxic. In the endothelial cells of tumor vasculature there is an enhanced activity of the enzyme alkaline phosphatase for which the disodium phosphate salts, and the phosphoramidates are substrates. The activity of alkaline phosphatase on the prodrugs clips off the phosphorous construct revealing the parent compound in cytotoxic form directly at the site of the tumor. The actual biological mechanism of cellular demise (in terms of cytotoxicity) is a binding of the parent compound to the colchicine site on β-tubulin resulting in an efficient inhibition of tubulin polymerization. Two of the prodrug constructs (Figure 1) that we have prepared demonstrate such remarkable selectivity of vascular targeting that it is now paramount to prepare these compounds in larger amounts for *in vivo* biological studies. In addition, an indole compound (Figure 1) that we have developed (in non-prodrug form at this point) is among the most potent (including both natural and synthetic) of all compounds currently known to bind to the colchicine site on β-tubulin (GI_{50} = 5.1 x 10^{-9} M (cytotoxicity against human cancer cells), and IC_{50} = 0.5 - 1.0 μM (inhibition of tubulin polymerization)), and it is now of the utmost importance to prepare the prodrug construct of this compound in large scale for *in vivo* toxicology and related studies.



Benzo[*b*]thiophene Prodrug (BBT-P)
Also called: VPM-V-152

Dihydronaphthalene Phosphoramidate

Indole Prodrug

Figure 1 Prodrug Constructs Selected for Scale-Up Synthesis

Specific Aims: The proposed scale-up syntheses will be accomplished by the following research strategy:

1) We have previously prepared approximately 25 mg of the benzo[*b*]thiophene prodrug (VPM-V-152), and the same synthetic route will be employed for the scale-up synthesis of this compound to the 1 g level.

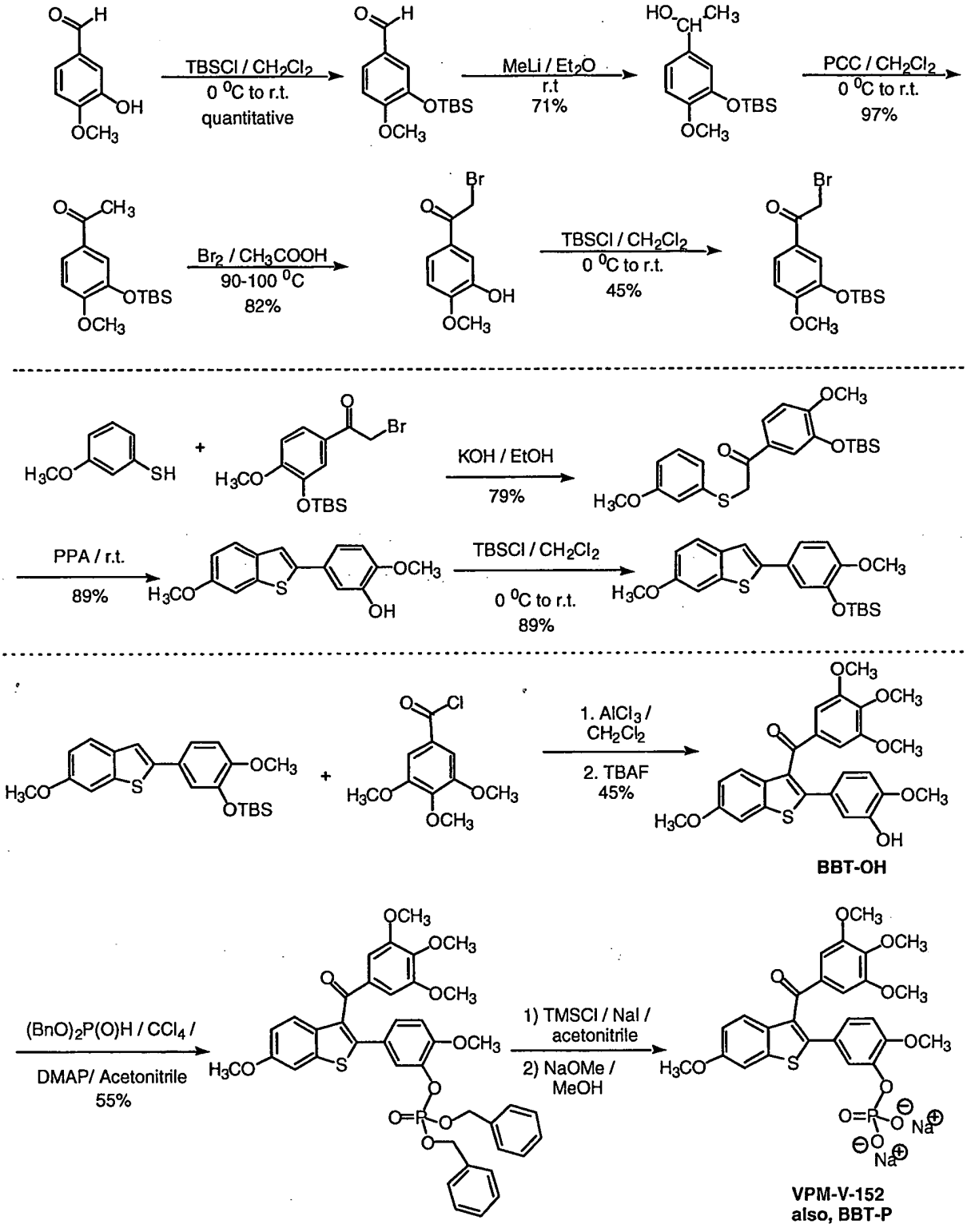
2) We have previously prepared approximately 20 mg of the dihydronaphthalene-based phosphoramidate prodrug, and we anticipate utilizing the same synthetic strategy that we have already developed in order to prepare 1 g of this compound.

3) To date, we have only prepared the indole compound without the *ortho* phenolic moiety and without the phosphate prodrug construct. The remarkable bioactivity of the non-prodrug construct warrants the immediate preparation of the indole prodrug in large scale to allow both *in vitro* as well as *in vivo* analysis. The synthesis has been modified accordingly in order to accommodate the phosphate moiety, and this route should allow the preparation of 1 g of the indole prodrug.

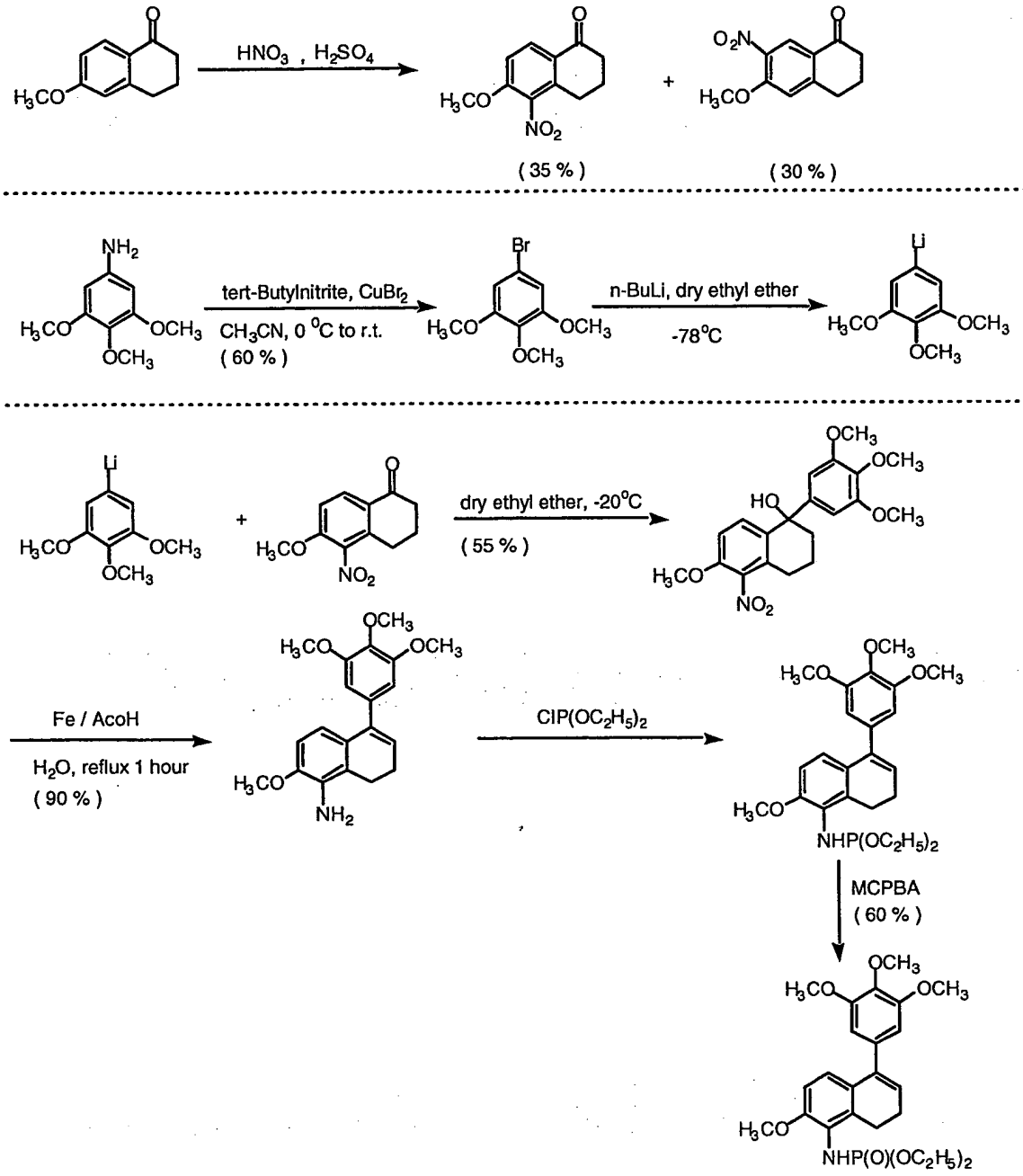
Research Design and Methods: The scale-up syntheses will be accomplished by the synthetic routes delineated in Scheme I (for the benzo[*b*]thiophene prodrug), Scheme II (for the dihydronaphthalene phosphoramidate prodrug), and Scheme III (for the indole prodrug). We anticipate that some of the slightly lower yields (on certain steps) obtained during the initial small-scale syntheses will be improved during the scale-up procedures. It may prove necessary to protect the secondary nitrogen of the indole prior to formation of the phosphate dibenzyl-ester. This issue will be addressed in due course.

Budget Justification: The budget for the project totals $ 11, 694 and is included with this proposal following the reaction schemes. The majority of the requested funds will be used to hire Mr. Tori M. Strong as a full-time research technician for three months (he is currently employed on a part-time basis). He has expertise in the proposed areas of synthesis, and will be invaluable in terms of bringing these projects to fruition in a timely fashion. The remainder of the requested funds ($4,000) will be used to purchase necessary glassware, chemicals, solvents, and related supplies. My research group currently includes seven graduate students, one postdoctoral research fellow, one research technician, and twelve undergraduate students. Several of these group members will be involved in various aspects of the syntheses outlined in Schemes I, II, and III.

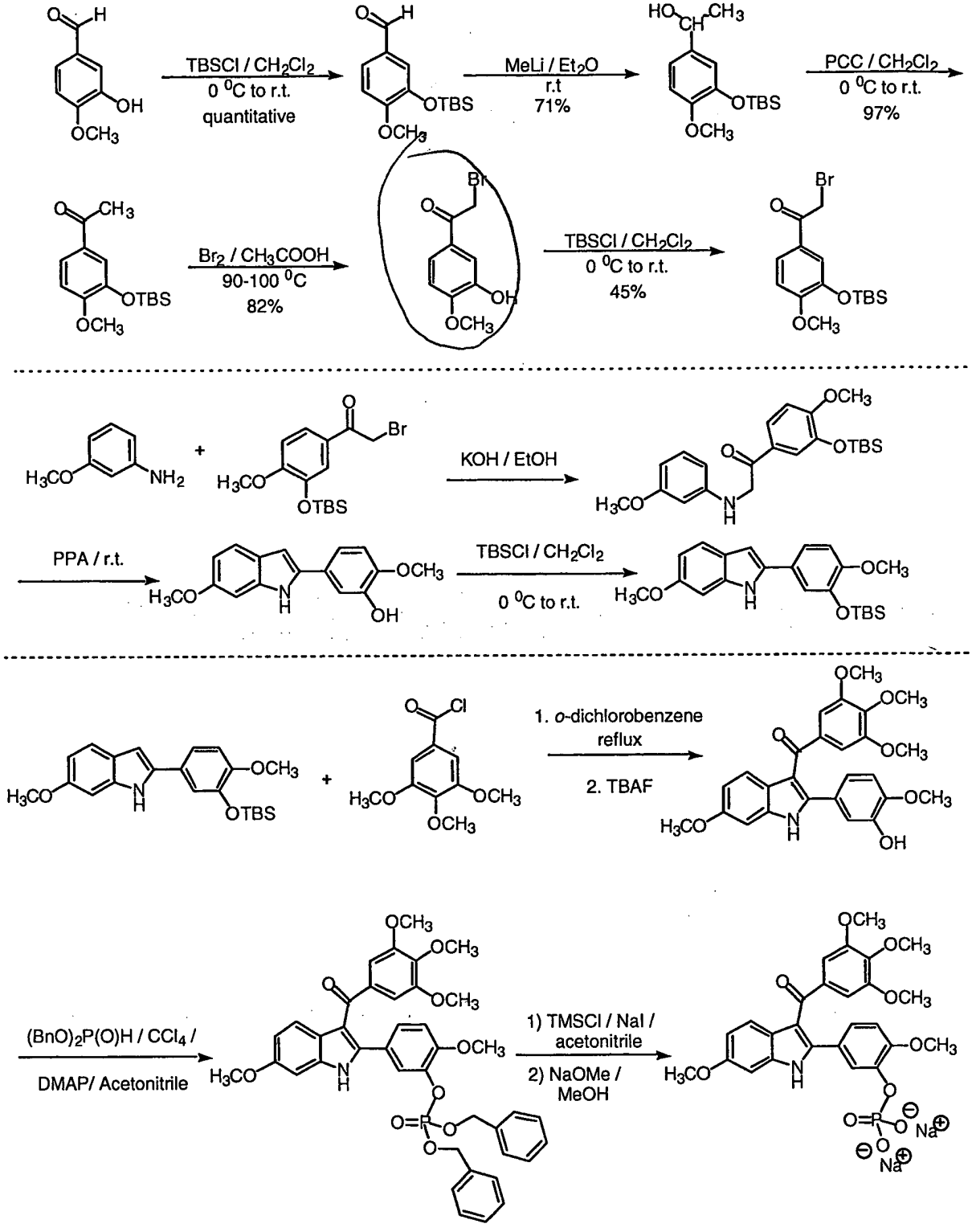
Scheme I. Synthesis of Benzo[*b*]thiophene Prodrug (VPM-V-152)



Scheme II. Synthesis of a Dihyronaphthalene Phosphoramidate Prodrug



Scheme III. Synthesis of Indole-based Prodrug



BUDGET REQUEST
PROJECT 2 SUPPLEMENT
SYNTHESIS OF TUMOR VASCULATURE TARGETING AGENTS

Professor Kevin G. Pinney. Principal Investigator

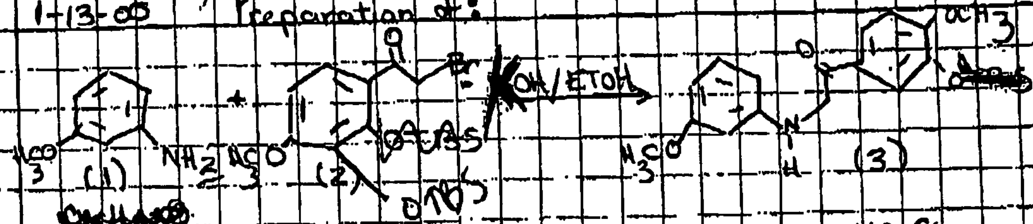
Salaries:		
Research Technician	three months @ 100% effort	$ 4,159
Total Salaries:		**$ 4,159**
Fringe Benefits:		
Research Technician	3 months (health, social security)	$ 1,248
Total Fringes:		**$ 1,248**
Supplies:		
Chemicals		$ 2,000
Glassware and disposables		$ 2,000
Total Supplies		**$ 4,000**
TOTAL GRANT COST (DIRECT)		$ 9,407
+ overhead (55% of salaries @$ 4,159)		$ 2,287
GRAND TOTAL		**$ 11,694**

Exhibit 6

(see attached)

Heather O'dell

1-13-05 Preparation of:



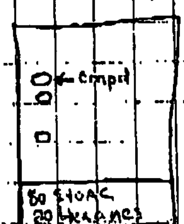
	(1)	(2)	(3)
	C_7H_8ON	$C_{15}H_{13}SiO_3Br$	$C_{22}H_{30}NO_4Si$
M.W	123.15	359.34	372.93
g	1g .912mL	2.92g	
mmol	8.18	8.18	
ratio	1	1	

Theoretical Yield .999g

Density = 1.096

Procedure

- A mixture of (16 ml) Ethanol and (8 ml) water is prepared
- KOH (1.5 g) is dissolved in it and stirred at 0°C under N_2
- m-anisdine (1g, 8.18 mmol) is then added (3:54 pm)
- followed by 1 equivalent of the Ketone (2) (2.92g, 8.18 mmol) (4:15pm) and stirred for 2 hrs (overnight)
- Work Up:
  - Add water (50 cm³)
  - Litmus = Blue
  - Add ethylacetate
  - $MgSO_4$ for 30 minutes
  - Rotovap

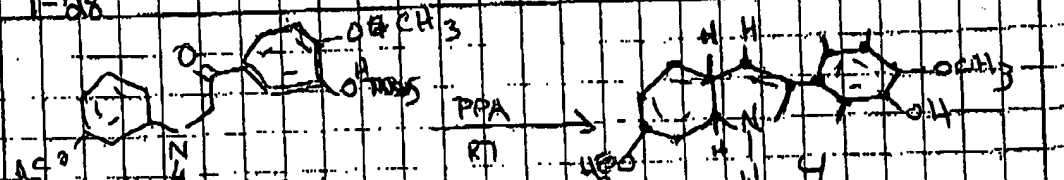


Ran a column
Ran NMR

Heather O'dell

9

1-58



PPA

$C_{10}H_{17}O_4N$ MW = 287 g/mol

$C_{16}H_{15}O_3N$ MW = 269.1

g ≈ g

mmol 3.48

ratio

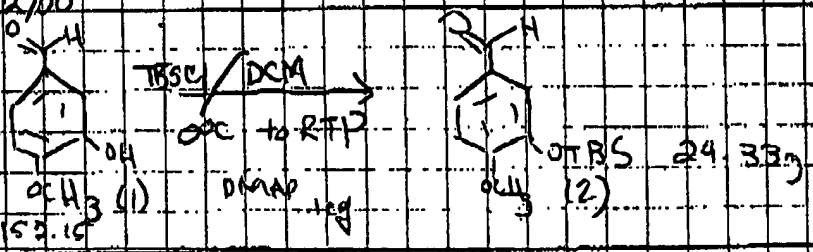
- Added CH_2Cl_2 to loosen product
- PPA was charged to a round-bottom flask @ 10 g (Inorganic
- at rt at 2:24 under N_2 (X10 PPA)
- Round bottom w/ 3 necks. Black grease on neck (drawer #4) + place stick in the motor
- Quench with H_2O at 3:24

- Workup
  - H_2O
  - CH_2Cl_2
  - $MgSO_4$

Heather O'dell

11

12/12/00



M_W = 152.15

0.1 mole = 15.2g

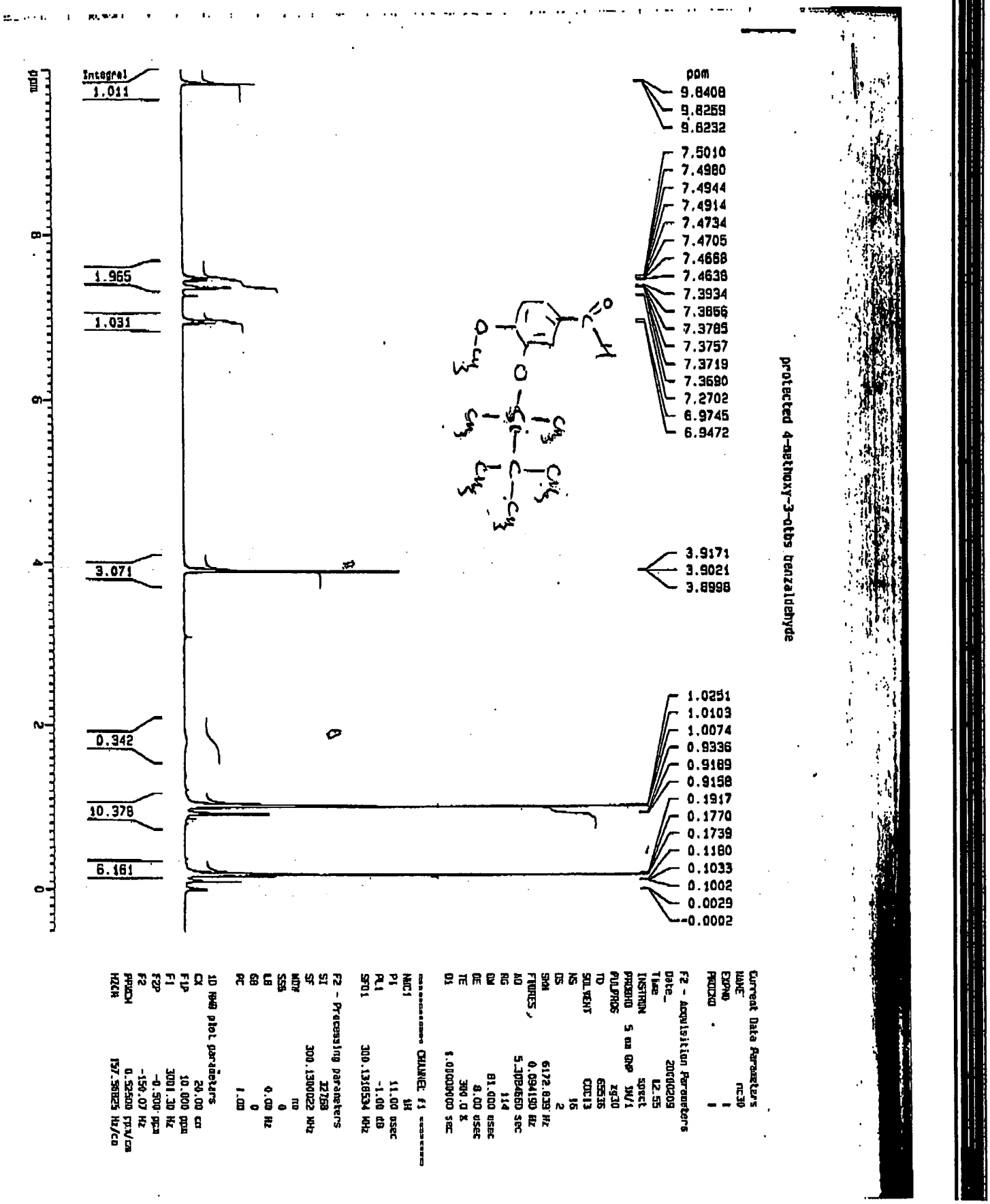
1:1 eq TBSCl
1:1 eq Et3N
1:1 eq DMAP

Procedure

3-hydroxy-4-methoxy (0.1 mole, 15.2g) were dissolved in dry DCM (100 cm³) and stirred at 0°C (ice bath). To the cold stirring mixture was added the 1:1 eq of triethyl amine (0.1 mole, 11.132g, 15.27 cm³) & 0.11 eq DMAP (0.011 mole, 1.344g) 1.1 eq of TBSCl (0.11 mole) was added and stirred overnight

Work up
- Gelled H_2O (Bottom Layer)
- $MgSO_4$
- Filter
- Rotovap

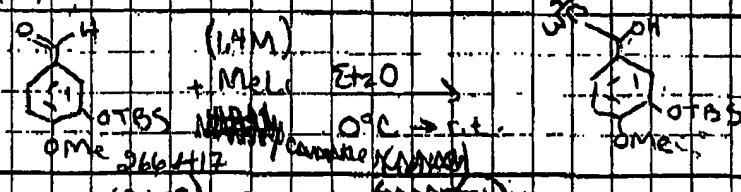
Heather O'dell



Heather O'dell

13

D-4



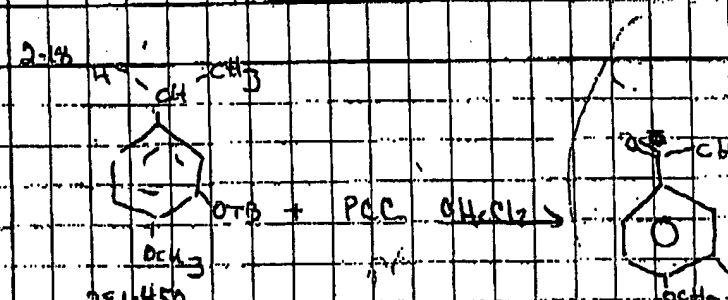
g	5.00 (14.33)	28.15 x 4	≈200 ml
mol	18.767	28.15	
ratio	1	2	

Amounts

* (P) - alcohol in Et2O in around 500 ml cooled in ice/under N2
* added MeLi - stirred at room temp overnight rt for an hr
* Work up: H2O, keep top layer * Rotavap
* Went directly to next step (p15)

Heather O'dell

15

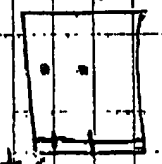


MW	281.450	215.56	280.443
g	24.12 g	27.71 g	25.18 g ?
mmol			
Ratio	1	1.5	
Weight	24.12	≈ 27.71	

Procedure

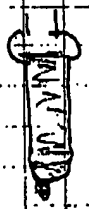
- Alcohol in ≈ 250 ml CH_2Cl_2 cooled cooled in ice/H_2O . Stirring under N_2
- Add PCC bring to rt 10:30 - 4:30
- Stirr for 4 hrs

TLC: 90/10 hex/sta Ac



Compared w/ Never ran higher than 80/20 Product is colorless

Never let column Dry!



Workup

Filter with celite & silica gel and wash with DMC

Rotovap the filtrate

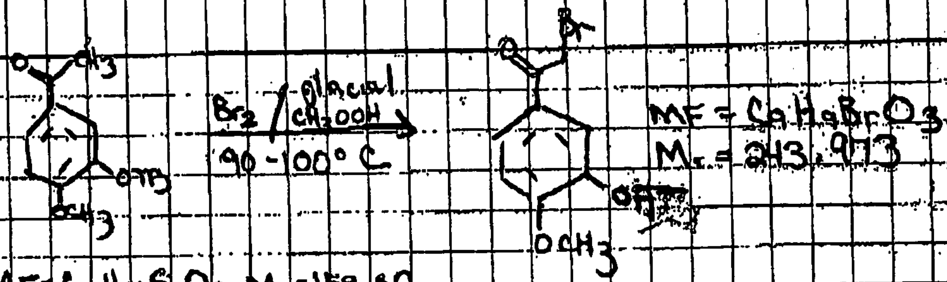
Flash Column loaded & eluted in 90/10 hex/EtOAc

- Silica w/ hexanes (can be crude) No bubbles?
- Put under pressure to push gel down
- Put silica gel in empd
- Flash column
- Sand on top of empd
- hexanes in a flask w/ left over empd
- Apply pressure w/ flask to catch extra

Heather O'dell

17

2-25



MF = $C_{11}H_{24}SiO_3$ Mr = 159.82
MW = 268.149 d = 3.102g

g		
mmol		
ratio	1	0.9

Procedure: • 800 ml AcOH heated to 90°-100°C

• Add ketone

• Stirr for 10 min

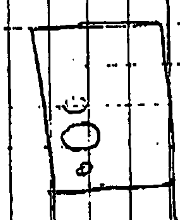
• Added Br_2 dropwise over 15 min

• Stirred at for 15 min

Work up: Ice Water
CH_2Cl_2 (3x)
Brine

Load
Start
GC: 250 final
Start 100°C
15 ml of solution
No

TLC 60:40

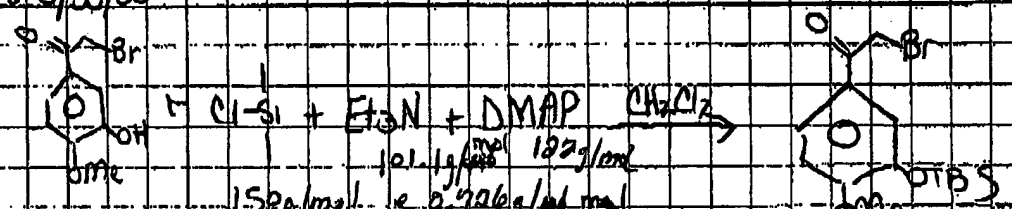


• Heater • By nitrogen by needles
• Acetic Acid under hood
• Magnetic Bar
Clean: Na thio sulfate + water?

Heather O'dell

29

9 5/30/00



mg	~~150~~ 9.16g	~~[illegible]~~ 10.98	~~[illegible]~~ 4.00	~~[illegible]~~ .44		
mmol	.036					
	1	1.2	1.52	0.1		anh

Procedure
- 3-hydroxy-4-methoxy (9.16g) dissolved in Dry DCM (100 cm³)
- Stirred at 0 °C
- To cold stirring Et3N (10.98 mL)
- Add DMAP (.44 g)
- TBSCl (6.48)
- Stir Overnight

- Workup
  - H2O
  - MgSO4
- Rotavap

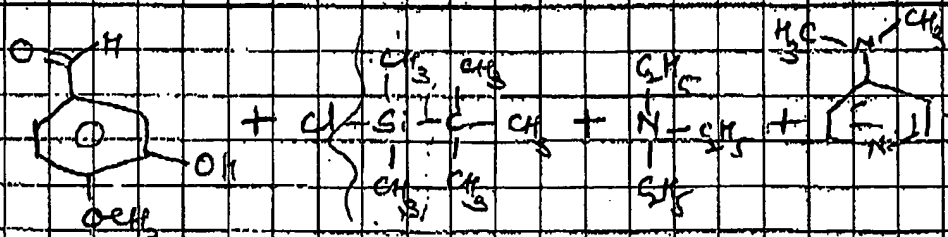
Column
- Elute in 90/10

Exhibit 7

(see attached)

Mallinath Hadimani.

6/15/00



	mbt-II	TBSCl	Triethyl amine	DMAP
MF	$C_8H_8O_3$	$C_6H_{15}SiCl$	$C_6H_{15}N$	$C_7H_{10}N_2$
MW	152	150.5	101	122
mol	0.1 mole	0.11 mole	0.11 mole	0.011 mole
Ratio	1	1.1	1.1	0.1
Gms.	15.2 gms	16.5 gms	11.132 gms. (d = 0.726 g/mL, 15.3 mL)	1.344 gms.

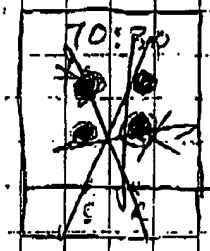
Ref: Nandeo [redacted] -I Page no. 60-61

Procedure:- 15.2 gms of 2-Hydroxy-4-methoxybenzaldehyde was dissolved in 300 mL of dry (distilled) dichloromethane (Dcm) and stirred at 0°C in an ice bath. To cold, stirring mixture was added 15.3 mL (11.132 gms) of triethylamine, 1.344 gms (0.011 mole) of 4-Dimethylaminopyridine (DMAP) and 16.5 gms (0.11 mole) of TBSCl. The solution was stirred at RT. overnight, under N_2. It was seen that the rxn. was complete in a 5-6 hrs.

6/19/00

Work up: Add about 300-400 mL of water, separate the organic layer, dry and evaporate to (by rotovap) to get the product as a oily thing creamy colored, oily liquid.

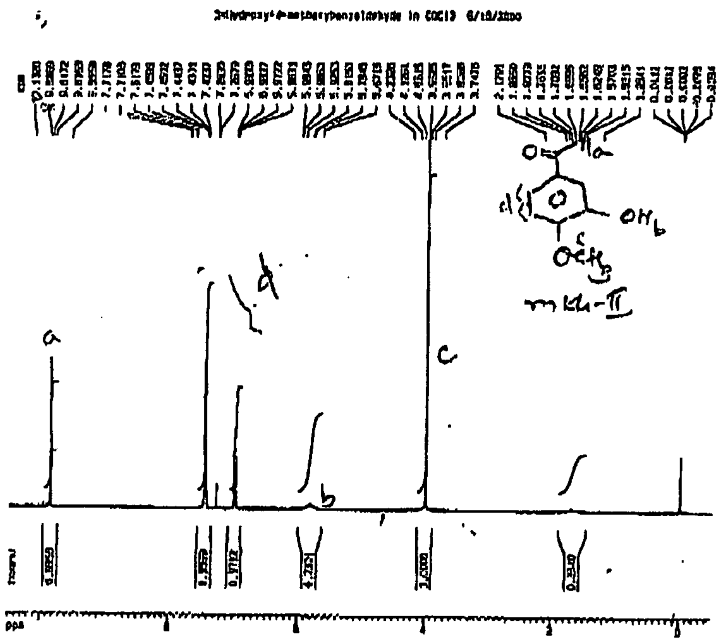
Result:

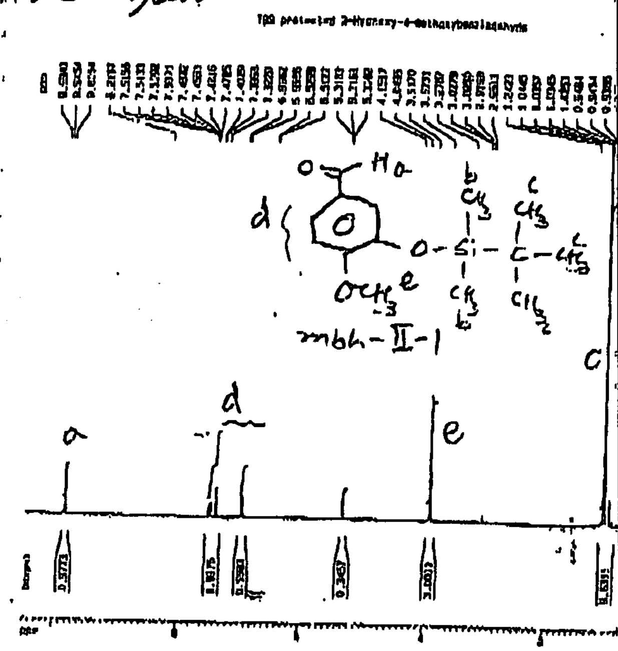


S → Starting compd (mbt-II)
R → Rxn. mixture (mbt-II-1)

~99% yield

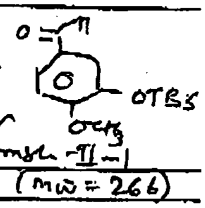
Took QC-MS and NMR after working

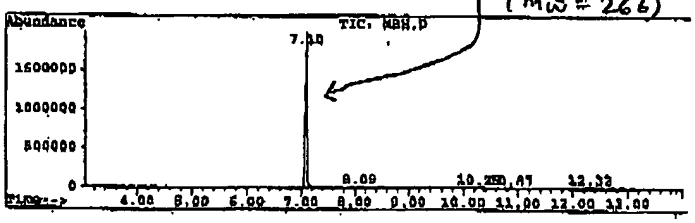




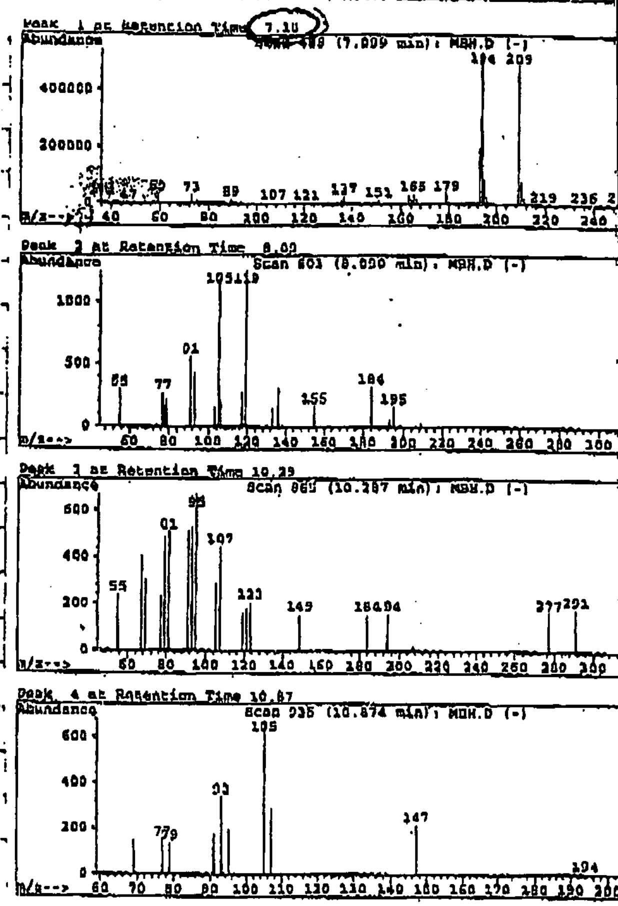
Area Percent Report -- Sorted by Signal

Information from Data File:
File : C:\HPCHEM\1\MBH.D
Operator : mbh
Acquired : 19 Jun 100 9:16 am using AcqMethod MBH
Sample Name: mbh-II-1
Misc Info : TBS protected
Vial Number: 1
CurrentMeth: C:\HPCHEM\1\METHODS\MBH.M





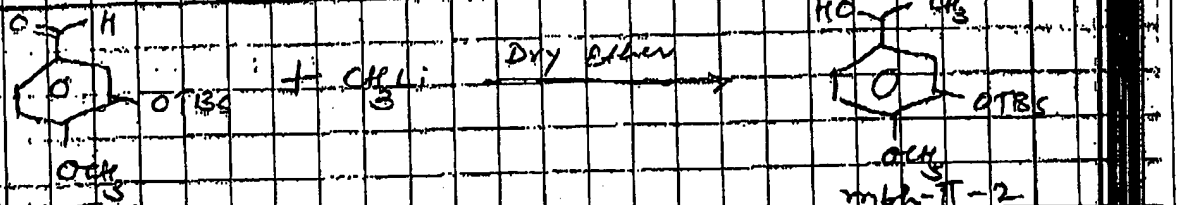
Retention Time	Area	Area %	Ratio %	Type	Width
Total Ion Chromatogram					
7.090	4089981	98.260	100.000	rBV	0.093
8.090	22601	0.543	0.553	rBV	0.118
10.287	15583	0.374	0.381	rBV	0.092
10.874	5559	0.134	0.136	rBV	0.092
12.317	28695	0.689	0.702	rBV	0.084



C:\HPCHEM\1\MBH.D Mon Jun 19 09:50:43 2000

70

Mallinath Hadimani



mbh-II-1 mbh-II-2

$C_{14}H_{22}O_3Si$ 266 — 0.1 — 26.6 gms

CH_3Li 22 — 0.21 — 2.1 — 4.62 gms (150 mL)

$C_{15}H_{26}O_3Si$ 282

Nandec chem-I, Page no. 62

Calculations:

# of mols of mbh-II-1 = 0.1 mol.

# of mols of CH_3Li = 0.1 × 2.1 = 0.21 mols.

i.e. # of gms of CH_3Li = 0.21 mole × 22 gms/mol

= 4.62 gms

For 1.4 m soln:

= 1.4 × 22 = 30.8 gms in 1000 mL Diethylether

∴ 4.62 gms in = 4.62 × 1000 / 30.8

= 150 mL

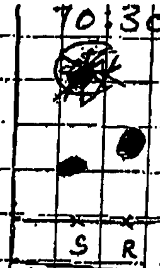
Procedure: 26.6 gms (0.1 mole) of protected aldehyde (mbh-II-1) dissolved in 250 mL of dry ether and cooled in a bath under N_2. Then, 150 mL (0.21 moles) of methyl Li in 1.4 m ether solution is added slowly, dropwise using a syringe or cannula over a period of 1 hr. The mixture is stirred at RT for about 10 hrs & over

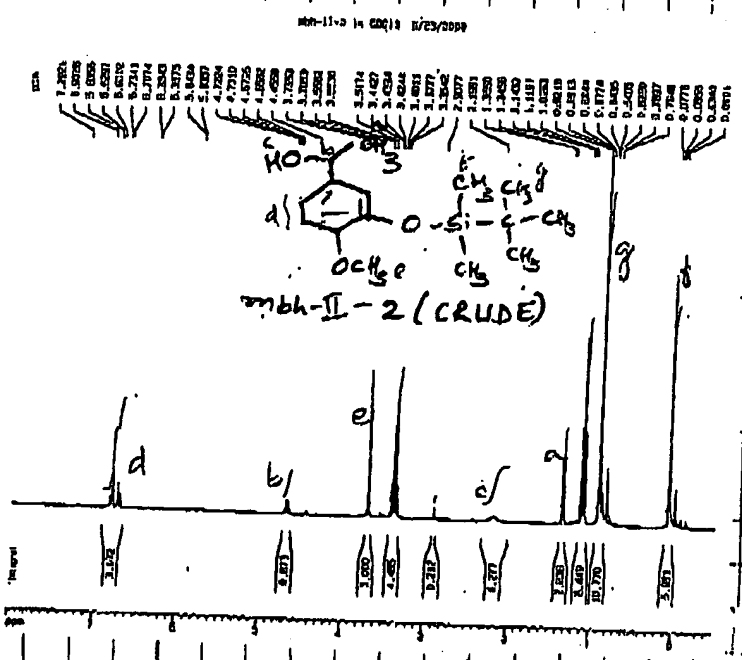
6/28/00

Workup: Add water, very slowly, dropwise & very carefully to destroy the excess of CH_3Li. Then it was extracted. Organic layer separated, dried over Na_2SO_4, filtered and rotovaped, to get the product as a light yellow liquid.

Results 70:30 Vijayawada Hazaribagh

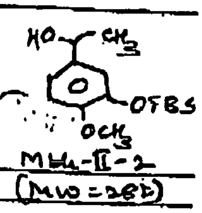
S → mbh-II-1 starting
R → Rxn. mixture mbh-II-2

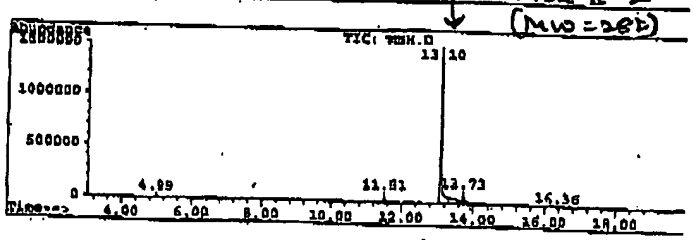




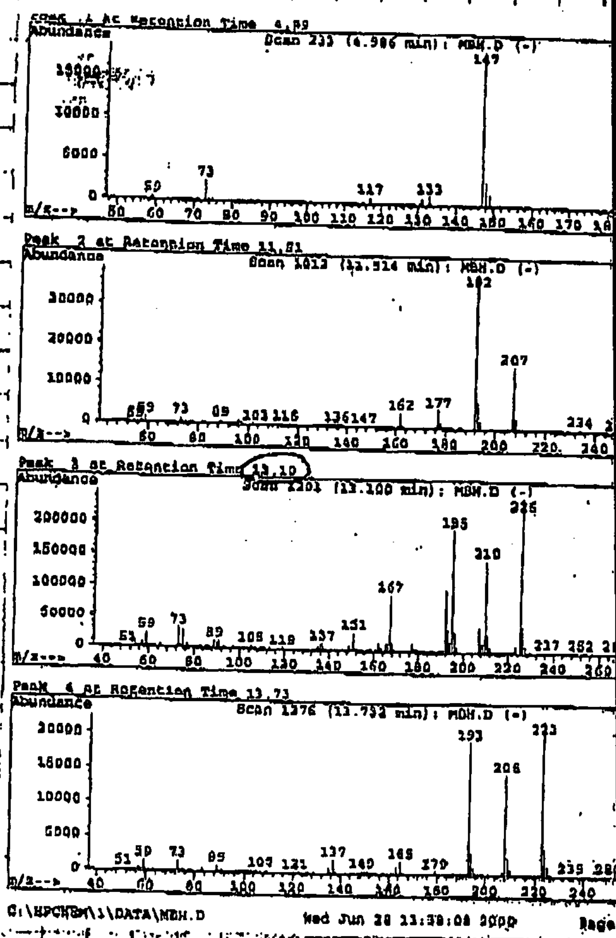
Area Percent Report -- Sorted by Signal

Information from Data File:
File : C:\HPCHEM\1\DATA\MBH.D
Operator : mbh
Acquired : 28 Jun 100 1:17 pm using AcqMethod MBH
Sample Name: mbh-II-2
Misc Info : With Methyl lithium
Vial Number: 1
CurrentMeth: C:\HPCHEM\1\METHODS\MBH.M



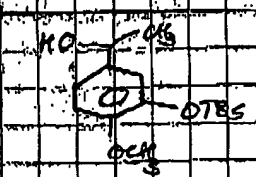


Retention Time	Area	Area %	Ratio %	Type	Width
Total Ion Chromatogram					
4.086	64268	1.477	1.666	rBB	0.084
11.514	218773	5.020	5.671	rVV	0.185
13.100	3857905	88.690	100.000	rBV	0.279
13.732	194151	4.463	5.033	rVB	0.152
16.350	14763	0.339	0.382	rBV	0.143

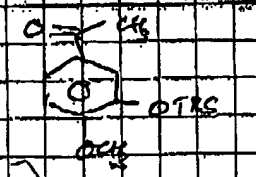


Mallikarjuna Hadimani

85



PCC
CH_2Cl_2
0°C to RT



mbh-II-2

$C_{15}H_{26}O_3Si$
282
0.0266
1
7.5 gms

PCC
$C_5H_5NHClCr_2O_7$
215.56
0.0292
1.1
6.3 gms

mbh-II-3

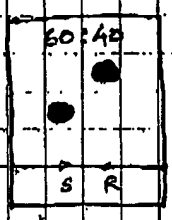
$C_{15}H_{24}O_3Si$
280

Nandeo Lab Book-I, Page no. 63

Procedure: 7.5 gms (0.0266 mmol) of mbh-II-2 was dissolved in 160 mL of dry (distilled) CH_2Cl_2 and cooled in an ice bath under N_2 atm. 6.3 gms (0.0292 mmol) of Pyridinium Chlorochromate (PCC) was added and the RM was stirred at RT for about 5-6 hrs.

6/29/00 Workup: Filtered the solid, water was added, Organic layer was separated. The separated organic layer was washed with brine and water 3 times. The water layer was extracted with CH_2Cl_2 3 times. Then, the combined organic layer was dried over Na_2SO_4, filtered and rotovaped to get the product as a brown liquid.

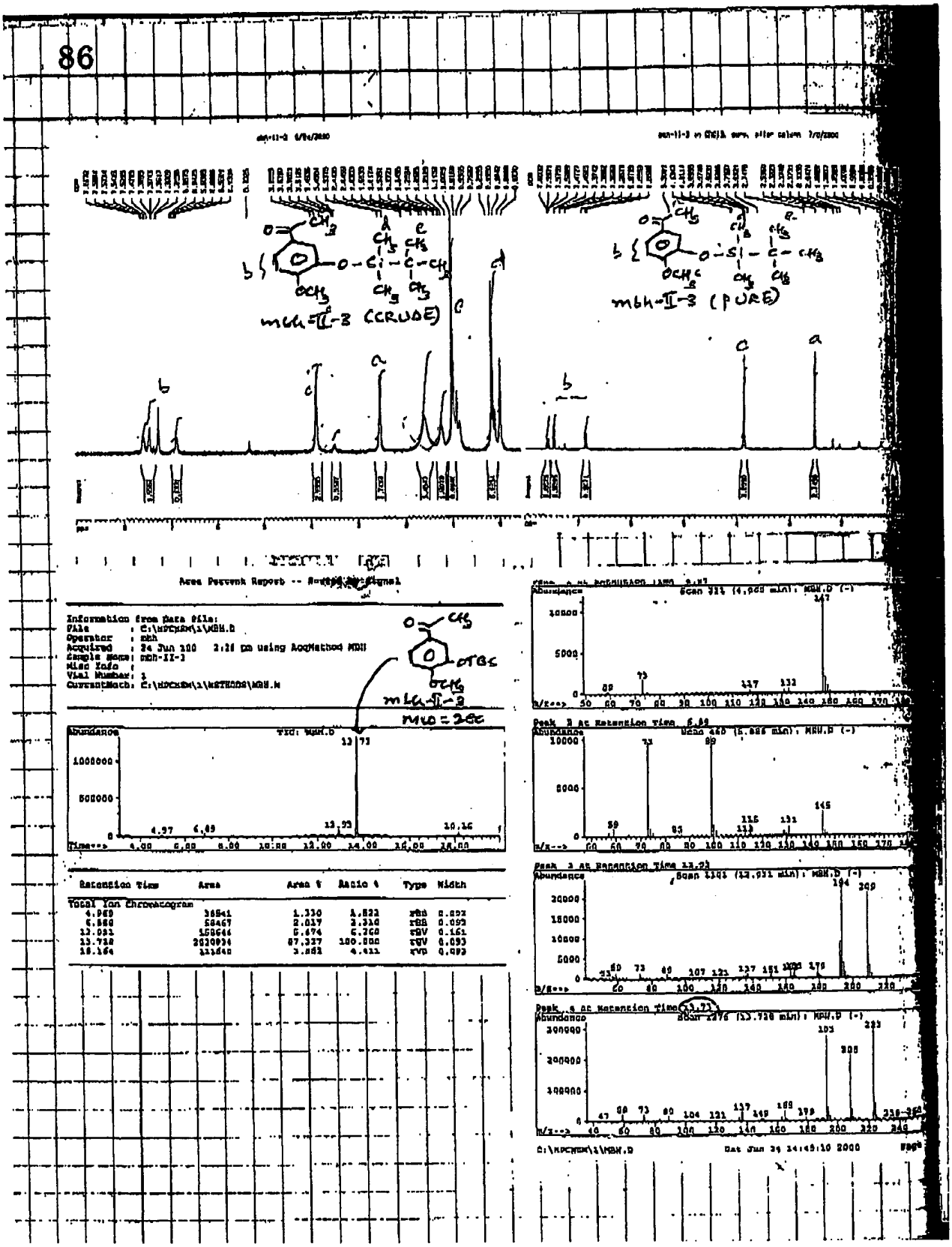
Result:



S → mbh-II-2
R → Rxn mixture (mbh-II-3)

Mallinath Hadimani

86



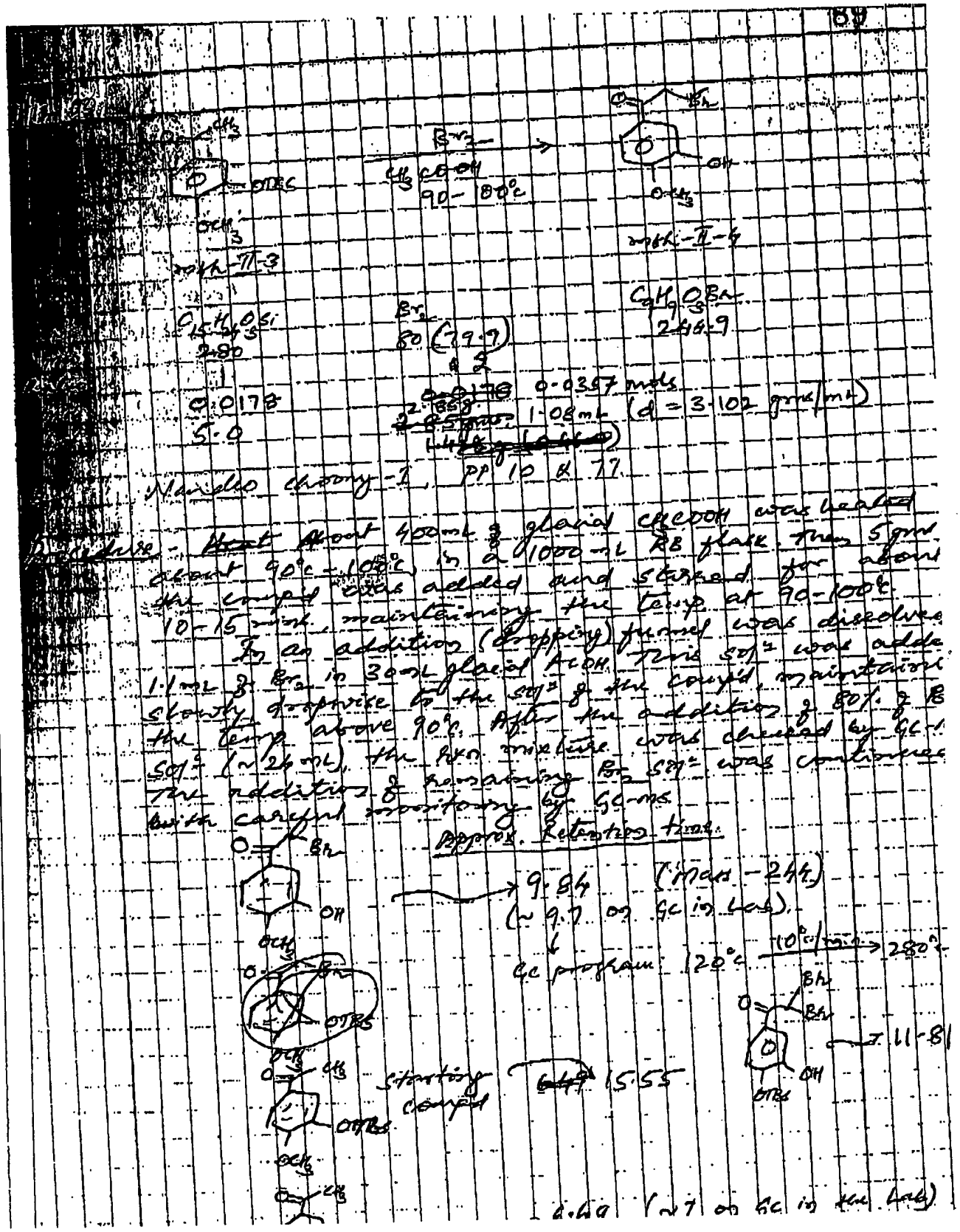
74

(6)

Mallinath Hadimani

Repeated the same procedure on P.no. 81
work up and separated the product
Repeated the procedure on P.no. 83. with product
obtained as from the first step.
work up
Repeated the same procedure on P.no. 91 with
0.2 moles (53.2 gms) of benzaldehyde
work up
Repeated the procedure on P.no. 83 with product
obtained on 6/24/00
work up
Repeated the procedure on P.no. 85
work up
7/6/00 Did a column and got fine yellow, needle-shape
7/6/00 crystals
* there was a problem with the column as the solve
was not coming out of the column. To overcom
mix the sticky compd with little silica gel and
spread it over the stationary phase
7/8/00. Took the NMR of the pure compd.
7/9/00 Continued Running the columns with the Reagen
product.
7/10/00 Was ready to go to next step but unfortunately
found out that heating material does not wor

Mallinath Hadimani



(8)

Mallinath Hadiyani

90

7/12/00

Work up:- * Add water
* Extract with CH_2Cl_2 (3 x 100-300 ml)
* Neutralise CH_2Cl_2 layer & $NaHCO_3$ sol.
* Extract $NaHCO_3$ layer & CH_2Cl_2 (2 x 100 ml)
* Collect all the CH_2Cl_2 layers

* Extract the aq. layer & Ethyl acetate (CH_3COOEt)
[i.e. the aq. layer after separating initial layers]
* Neutralise EtOAc layer & $NaHCO_3$ sol.
* Extract $NaHCO_3$ layer & EtOAc (2 x 100 ml)
* Combine all EtOAc layers.

* Mix the combined extracts of CH_2Cl_2 & EtOAc
* Dried over Na_2SO_4
* Rotavaped.

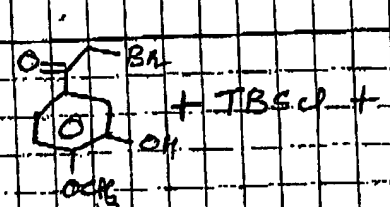
Took the NMR and GC-MS, looked very messy decided to run a column.

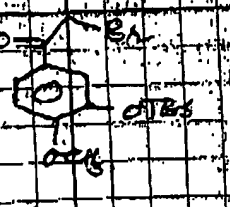
7/13/00 Did a column first & 90:10, then 80:20 & finally 70:30
Took NMR and GC-MS of 70:30 fractions, looked good ok and decided to proceed.

SEE P NO. 92 FOR NMR & GC-MS

(9)

Mallinath Hadingani





MBh-II-4

MBh-II-5

	$C_9H_{18}O_3Br$	$C_6H_{15}SiCl$	C_5H_5N	C_7H_6O	$C_{15}H_{23}O_3SiBr$
MF					
MW	249.9	150.5	101	122	359
Ratio	1	1.1	1.1	0.1	
mol	0.0099	0.01	0.01	0.00099	
Grms.	2.4299	2.7g	1.01g	0.216g	

(2.5 ml) $\lambda = 0.724$ g/ml

Dry CH_2Cl_2 ~ 50 mL

Procedure:- See P no. 81 of this book

* Continued the Rxn. for upto 36 hrs as there does significant peak on GC-MS

7/16/00 Work up:- See P no. 91 of this book. Took the NMR & GC-MS, & looked nicely. So decided to do a column.

7/17/00 did a column 1 90:10 and took NMR and GC-MS.

* NMR indicated that there carried to MBh-II-3 as an impurity. I decided to proceed ahead, after it will be a test & experience for the next step.

FOR NMR See P no. 94

7/18/00 Repeated the bromination Rxn. on P no. 89, with the following quantities

MBh-II-3 → 10.0 gms

Glacial CH_3COOH → 800 mL

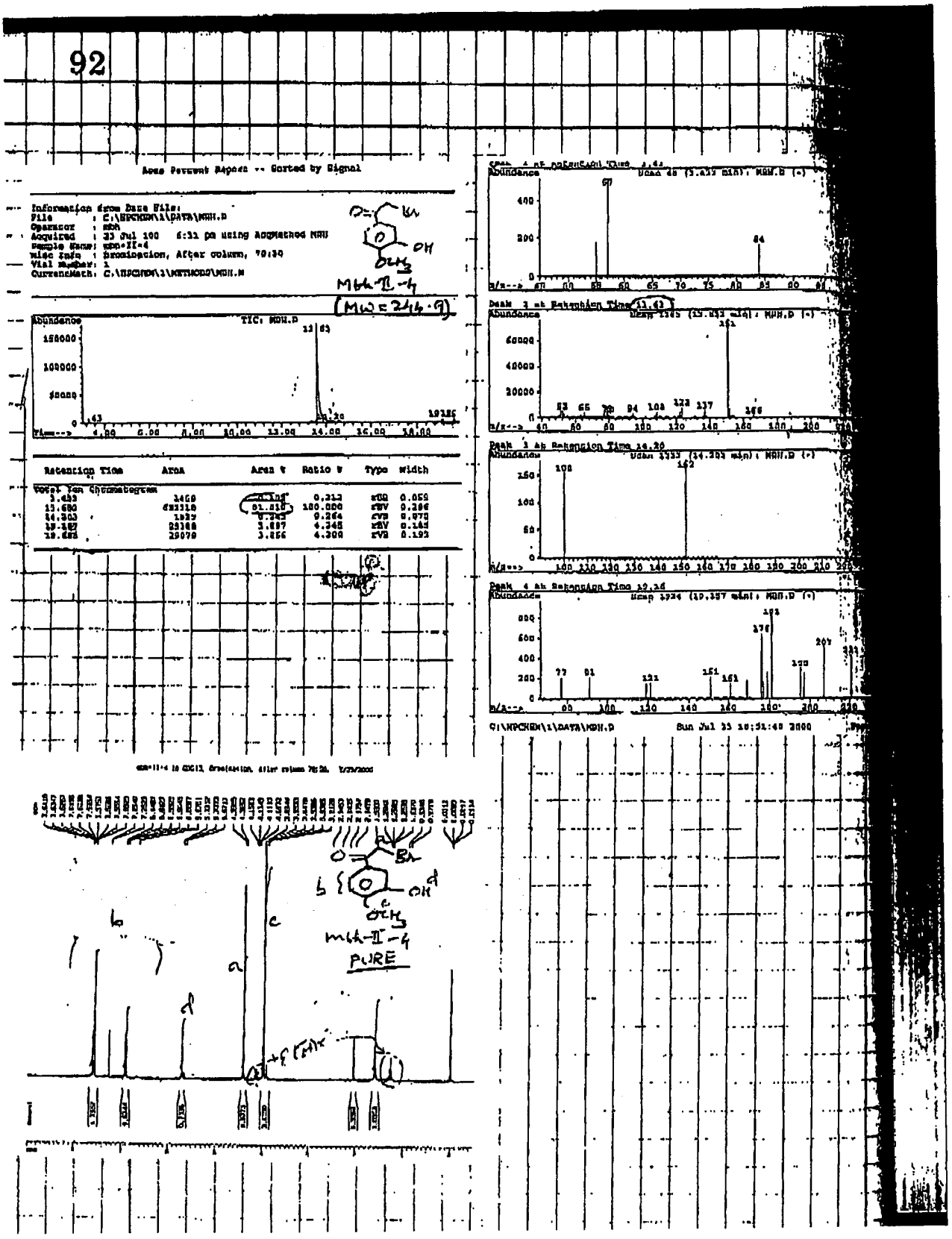
Br_2 → 2.2 mL in 50 mL AcOH

7/19/00 Did the work up as in P no. 90

7/21/00 Did a column, first 1 90:10, then 85:15 and finally 70:30. Column continued for 7/22/00 and 7/23/00 Took NMR and GC-MS of 70:30 fractions, and

Mallesath Hadimani

92

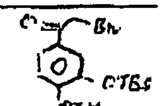


11

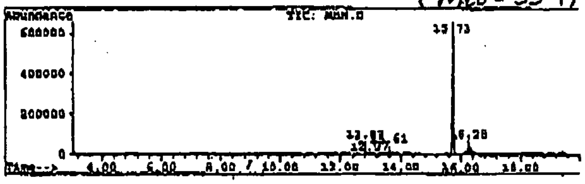
Mallesh Hadimani

Area Percent Report -- Sorted by Signal

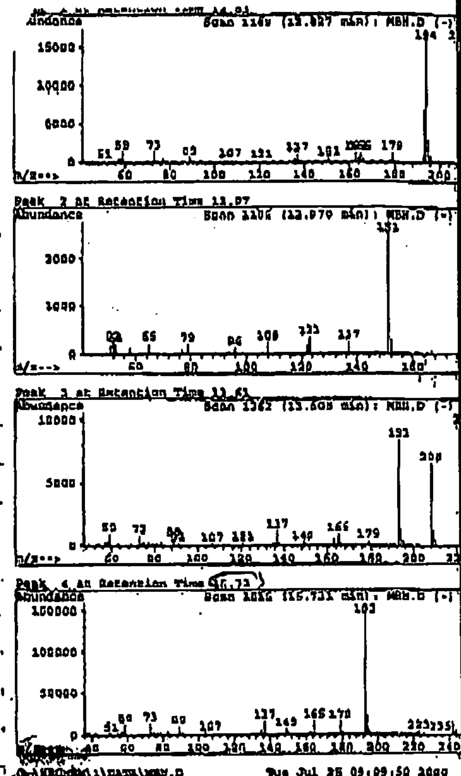
Information from Data File:
File : C:\HPCHEM\1\DATA\MBH.D
Operator : mbh
Acquired : 25 Jul 100 8:49 am using AcqMethod MBH
Sample Name: mbh-II-5
Misc Info : After 2.5 hrs., before work up
Vial Number: 1
CurrentMeth: C:\HPCHEM\1\METHODS\mbh.m

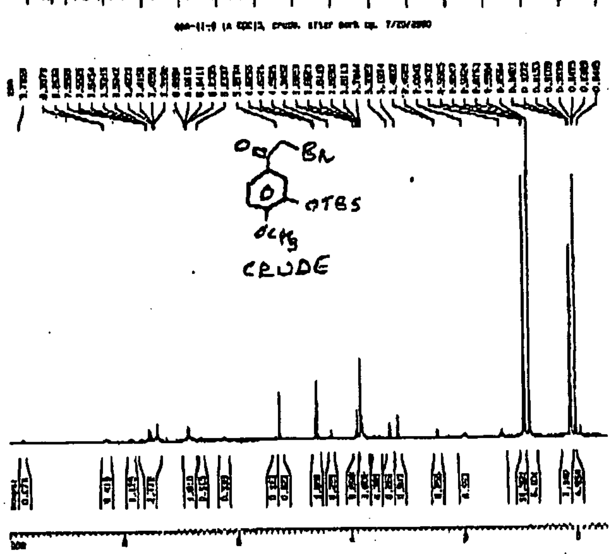


MbH-II-5
(Mw = 359)



Retention Time	Area	Area %	Ratio %	Type	Width
Total Ion Chromatogram					
12.827	112260	6.086	7.610	rBV	0.109
13.070	35782	1.840	2.426	rBV	0.151
13.608	83123	4.406	5.572	rVD	0.126
15.731	1475100	79.971	100.000	rBV	0.177
16.277	135316	7.567	8.437	rBV	0.093





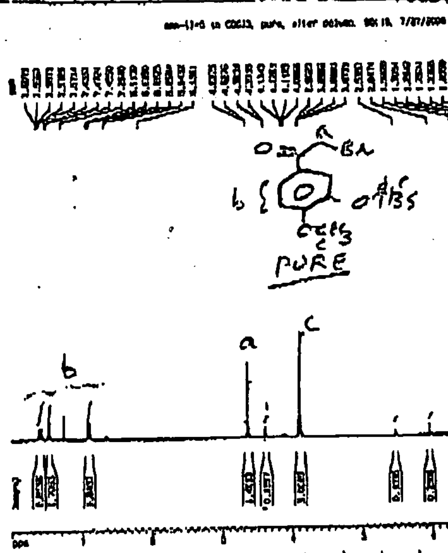


Exhibit 8

(see attached)

121.18 C6H5-N(CH3)2 d=.956 appreciate

Special 30 Day Project

1.096

Operation
MAJCK

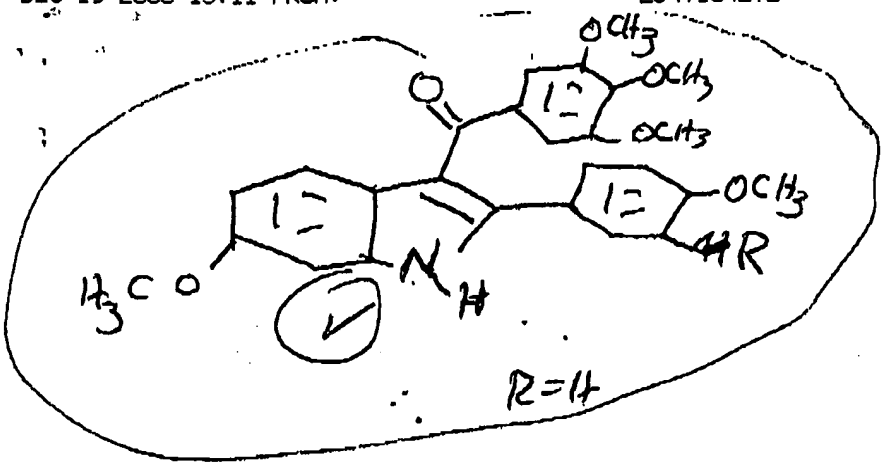
Heath
Malli
Jimmy
Anu
Chuck
Kevin

MJACK

MAJCK

Beginning 3:26 PM
~~October~~ September 23, 2000

754-2335
751-1636



Feng Wang

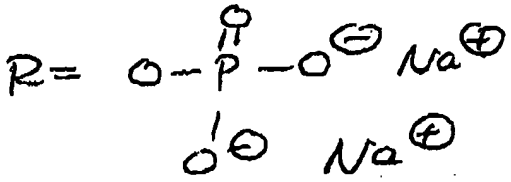
IC_{50} 0.5 uM

$GI_{50} \sim 10^{-9}$ M

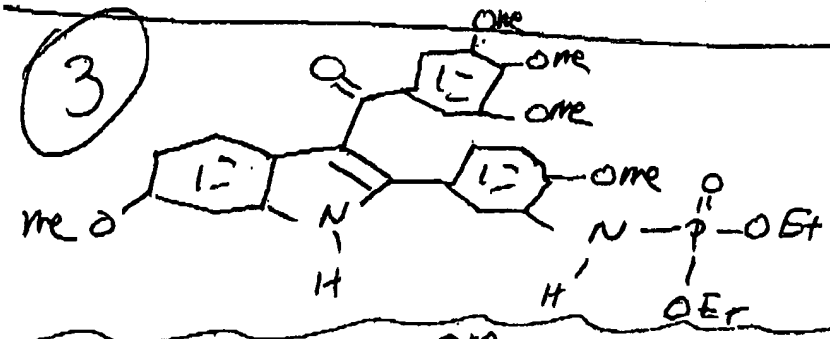
nM

1

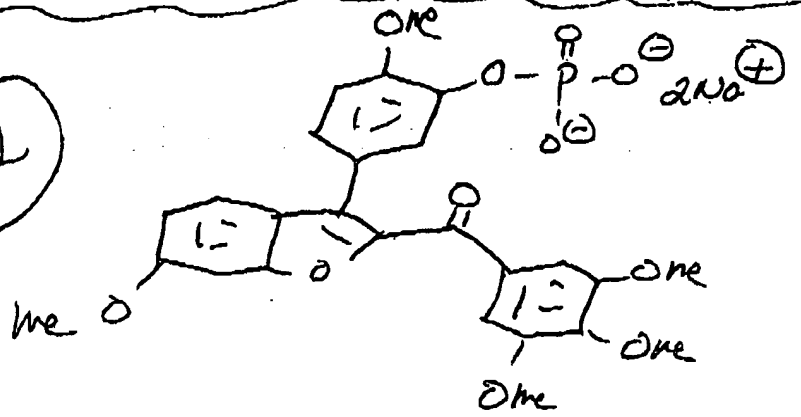
R=OH



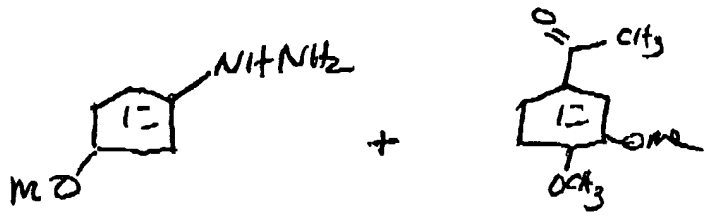
3

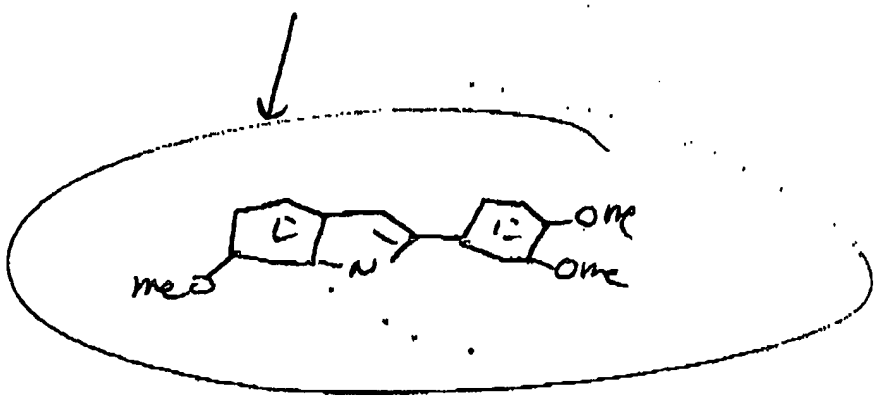


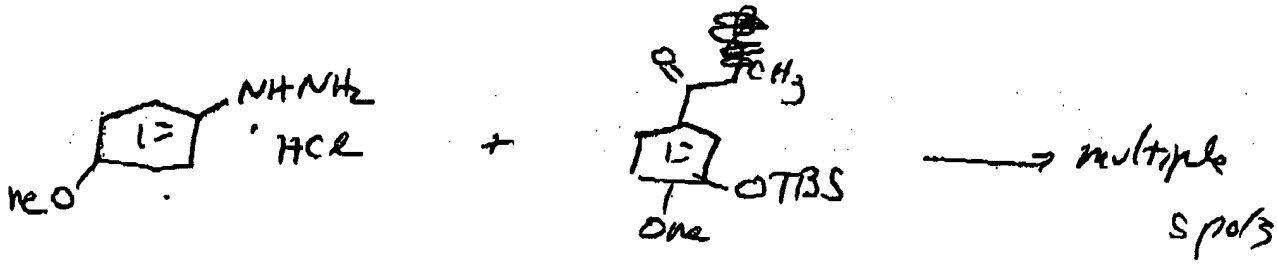
2



335



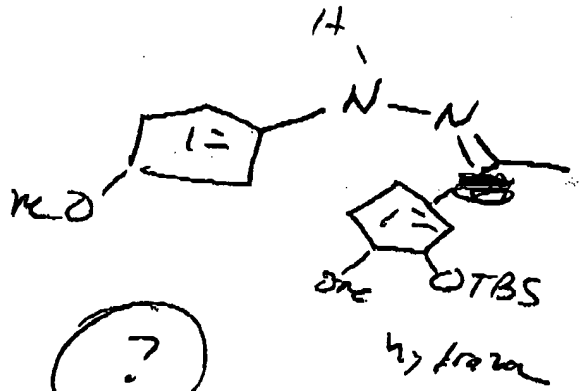




Dr. Carver

~~Free base~~

Free Base



?

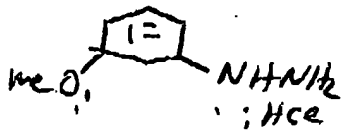
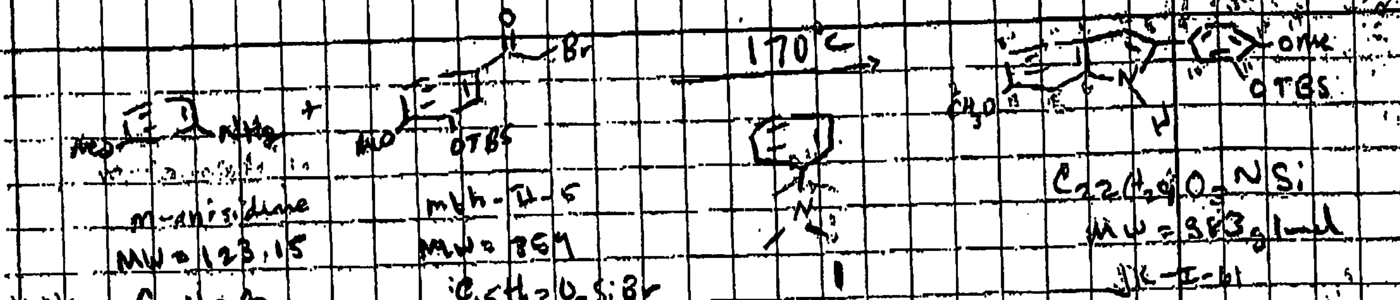


Exhibit 9

(see attached)

Indole Reaction 9/24/02 61



Ratio = 3 / 1
mol = .0042 mol / .0014
grams = .516 g (d = 1.096) / .5 g (1.40 eq)
mL = .47 mL (.75 mL)
= .5 mL / .44 / 1.3 mL

.184 g
.00223 mols
.480 mmol

Ref: Thesis of Feng Wang, Pg (56)

Procedure:
To a boiling mixture of .5 mL of m-anisidine and 1.3 mL dimethylaniline was added .5 g of mth-II-5 (in 1 mL EtOAc) slowly w/ a syringe. The rxn mixture was kept at reflux (170°C) for 2 hrs monitored by TLC

Work up: - EtOAc was added
- Extracted with EtOAc, brine
- dry with Na_2SO_4

TLC after I_2 stain

S_1 - m-anisidine
S_2 - N,N-dimethylaniline
S_3 - bromide

- when product is spotted a blue fluorescent is present under long UV

TLC in 60/40

1

62

Column: hexane ← dimethylformamide, 95%/5%, 90/10 ← spot above product; product mixture (above m-anisidine & product)

part of B

turns green under vanillin stain

A1 – product
A2 – 5 more than B
A3 – NMR taken
B1 – NMR taken
B2 – product (more)

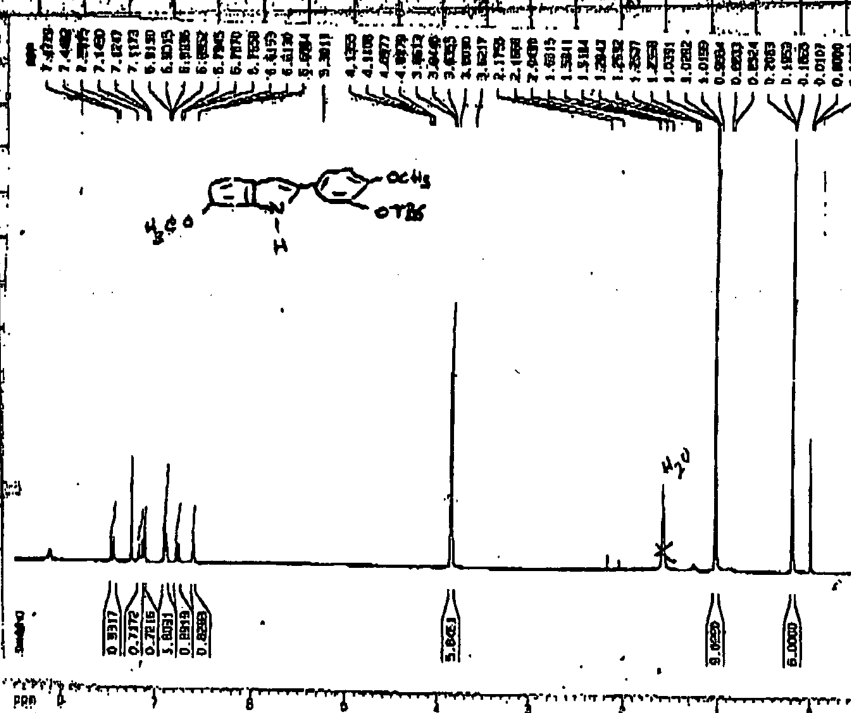
TLC after column

B1,2 – combined! after the column – orange color solid ⟹ purified by recrystallization hexane with crystals

% yield

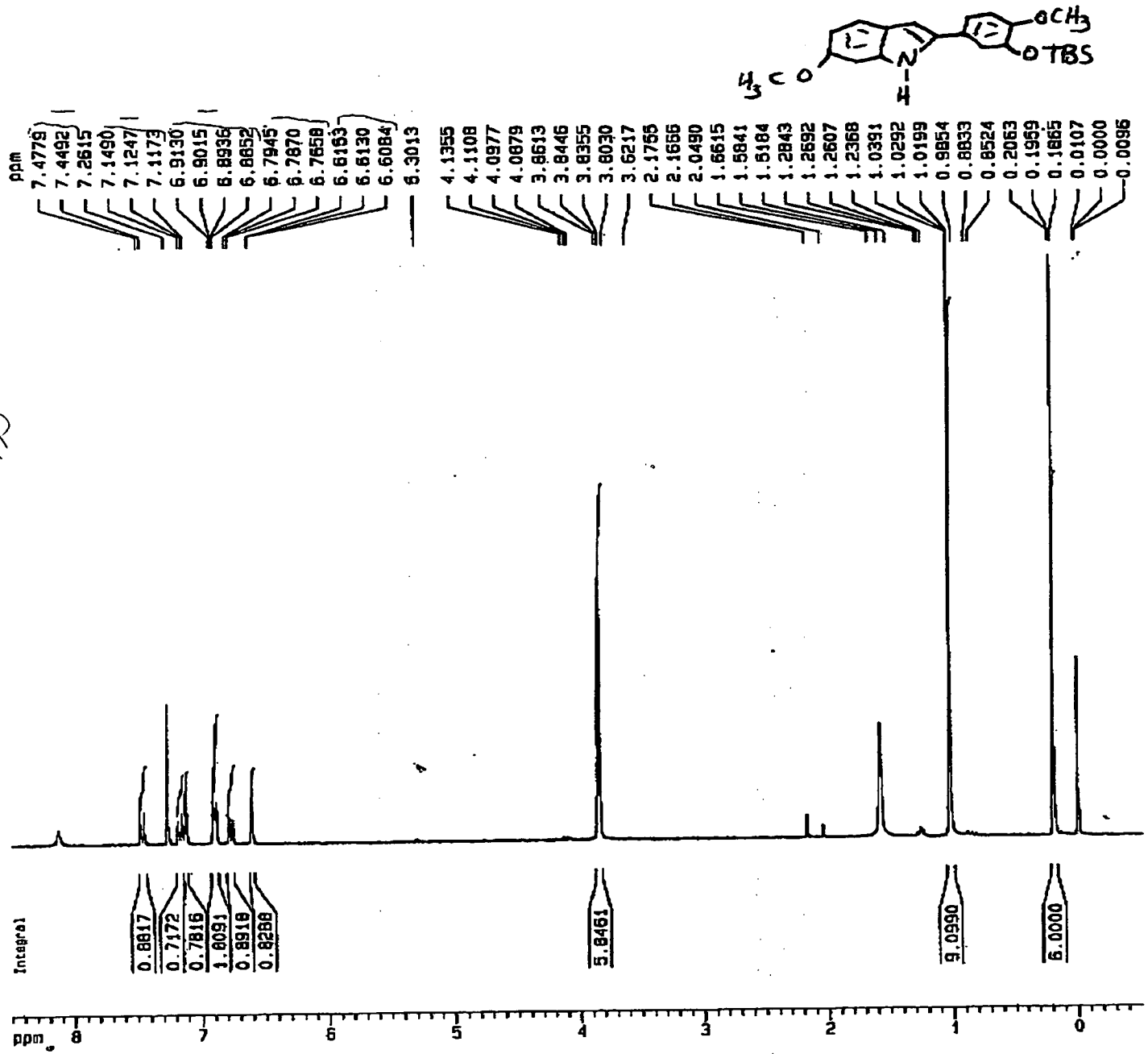
.0772 / 8.31 / .1042

actual = .1042
= .272 mmol 20%



(2)

88



orange band in column

Current Data Parameters
NAME junk
EXPNO 1
PROCNO 1

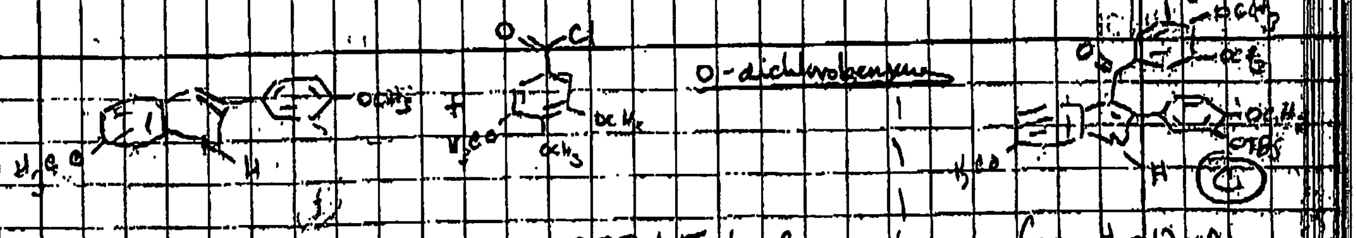
F2 - Acquisition Parameters
Date_ 20001003
Time 15.05
INSTRUM spect
PROBHD 5 mm QNP 1H
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 6172.839 Hz
FIDRES 0.094190 Hz
AQ 5.3084660 sec
RG 574.7
DW 81.000 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec

CHANNEL f1
NUC1 1H
P1 11.00 usec
PL1 -1.00 dB
SFO1 300.1316534 MHz

F2 - Processing parameters
SI 32768
SF 300.1300060 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

1D NMR plot parameters
CX 20.00 cm
F1P 8.500 ppm
F1 2551.10 Hz
F2P -0.500 ppm
F2 -150.07 Hz
PPMCM 0.45000 ppm/cm
HZCM 135.05850 Hz/cm

Model Reaction



MW = 253 g/mole M.W = 230.65 g/mole $C_{26}H_{25}O_6N$

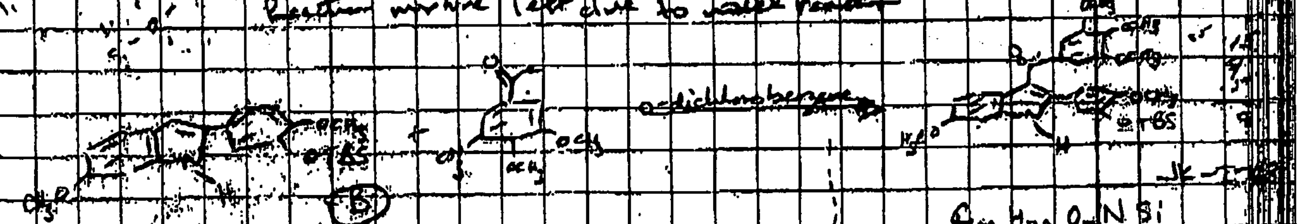
$C_{16}H_{15}O_2N$ $C_{10}H_{11}O_4Cl$ mw = 447

1 eq 1.5 eq 10 ml
.1 g .1367 g
.395 mmol .592 mmol

To a well sol of indole (.1 g, .395 mmol) in o-dichlorobenzene (10 ml) was added trimethoxy benzoyl chloride (.1367 g, .592 mmol). The reaction was refluxed for 12 hrs.

Workup - o-dichlorobenzene was removed by distillation under reduced pressure

Reaction mixture left due to watch reaction



$C_{32}H_{39}O_7NSi$

MW = 230.65 g/mole mw = 549

.1 g 2 .09 g .290 mmol
mmol .522 mmol .3916 mmol 1.044
1 eq 1.5

To a well sol of indole in o-dichlorobenzene was added 3,4,5-trimethoxybenzoylchloride. The reaction was reflux at for 48 hrs.
Work up - o-dichlorobenzene was removed as above.

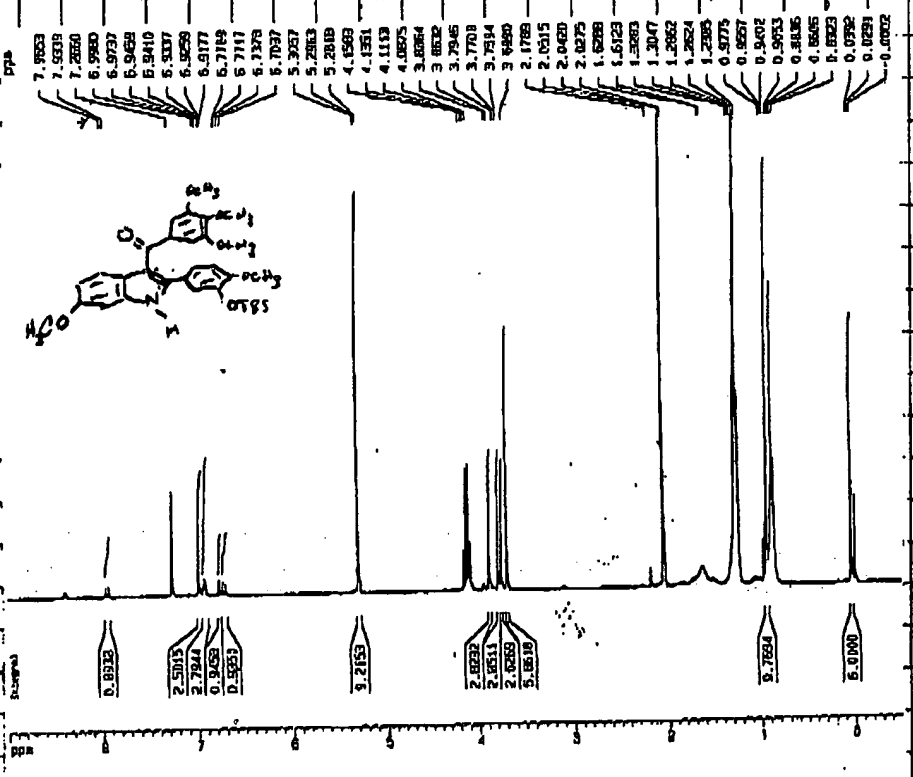
64

Column done previously

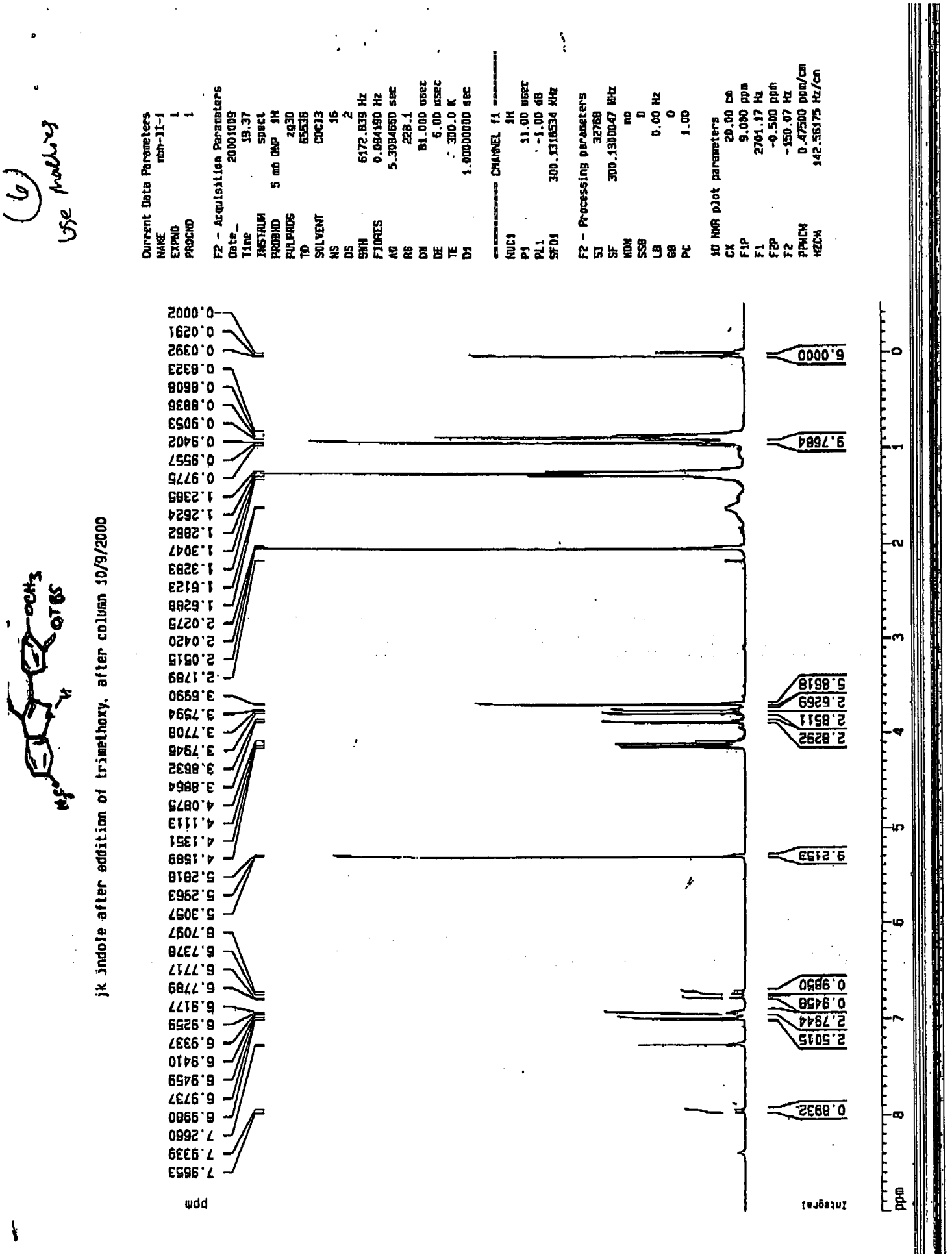
1st - 90/10 - hexane

2nd - 80/20 / 70/30 - spot where product and product came off

- a second column was done to separate these compounds



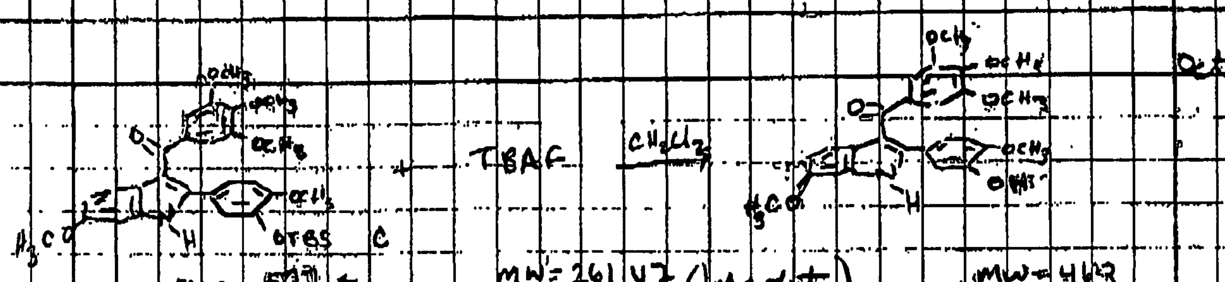
(6)
Use marking



(6)

03

Oct 19 [illegible]



MW = 577 ; MW = 261.47 (1M solution) ; MW = 463 ; IK-I-65

.14 g ; .0989 g ~ (.104 ml)

.090 mmol ; .135 mmol

1 eq ; 1.5 eq

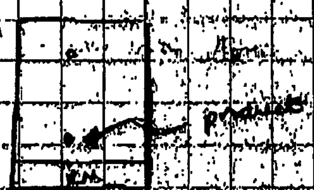
Procedure:

~~The [illegible] were added to the [illegible] (C) and the reaction was cooled to 0 °C. [illegible] solution of TBAF in THF was added to the reaction mixture. The reaction proceeded @ rt for 1½ hr.~~

Workup - H_2O

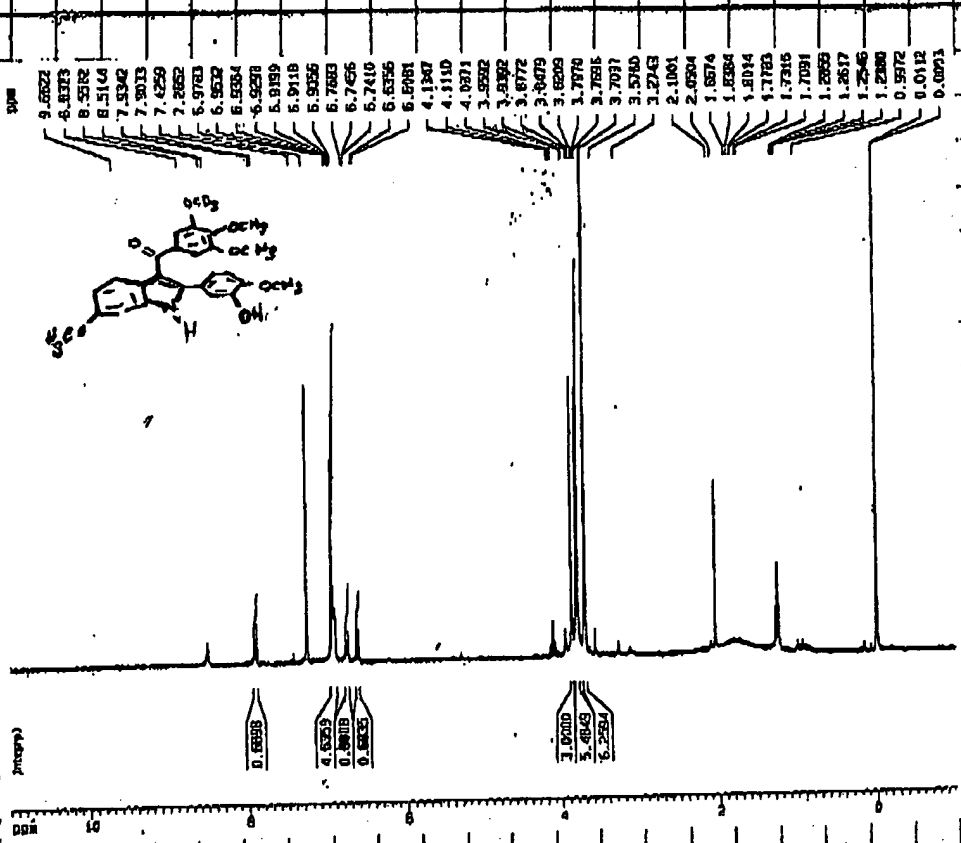
extract aq layer with CH_2Cl_2 (3x)

Na_2SO_4



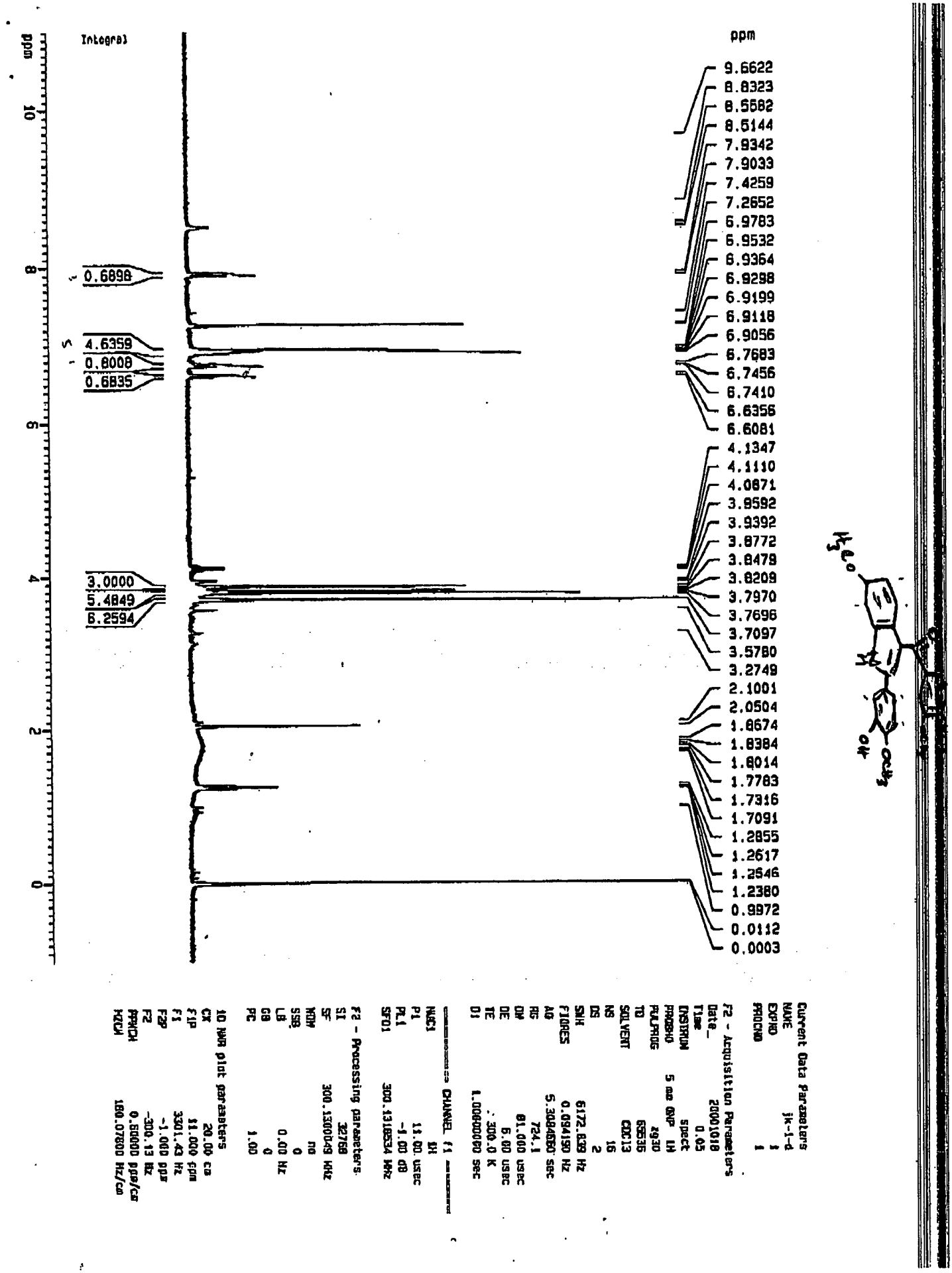
- column using 50/50 EtOAc/Hep.

66



93

(8)



Current Data Parameters
NAME jk-1-4
EXPNO 1
PROCNO 1

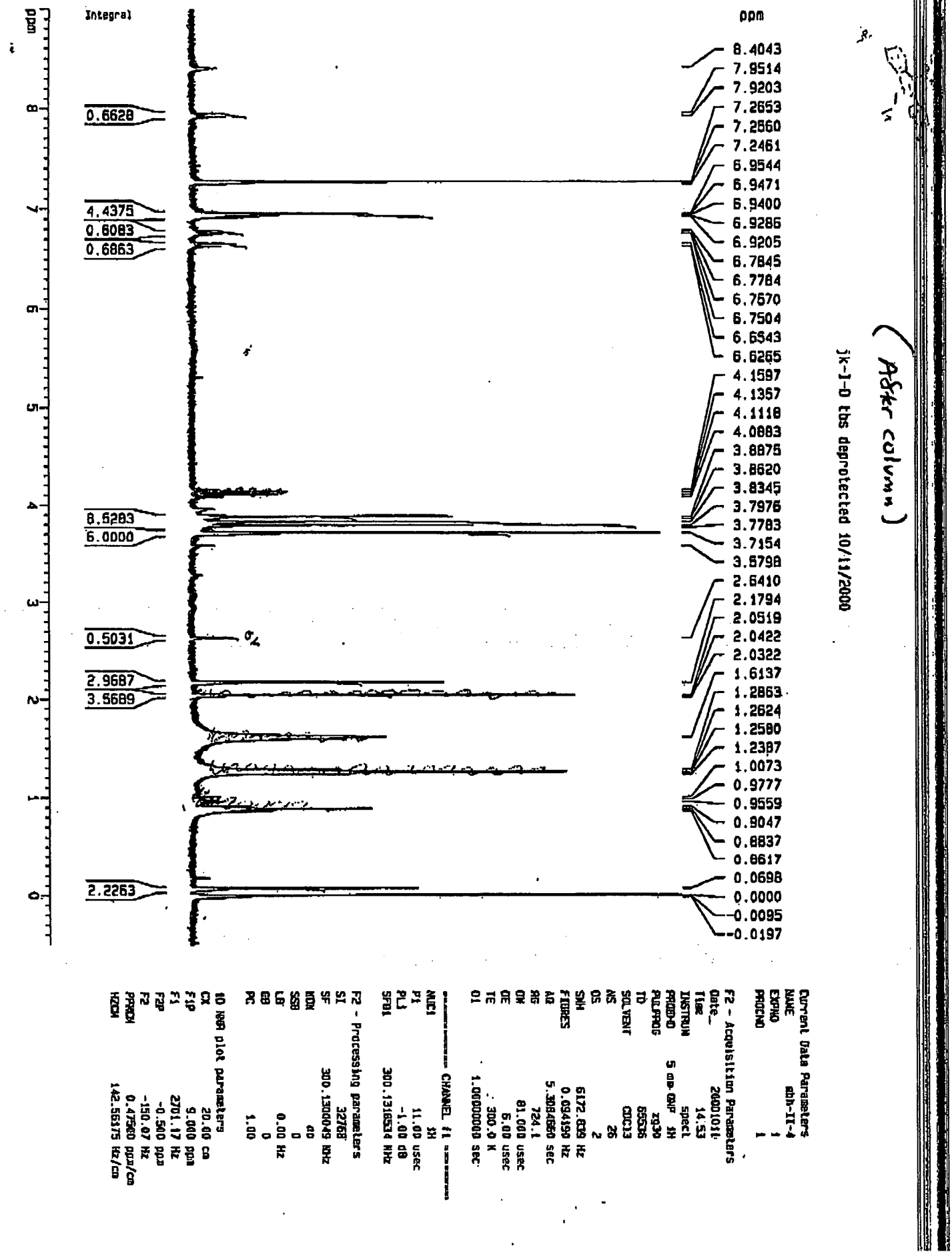
F2 - Acquisition Parameters
Date_ 20031018
Time 0.05
INSTRUM spect
PROBHD 5 mm QNP 1H
PULPROG z930
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 6172.839 Hz
FIDRES 0.094190 Hz
AQ 5.3084660 sec
RG 724.1
DW 81.000 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 11.00 usec
PL1 -1.00 dB
SFO1 300.1318534 MHz

F2 - Processing parameters
SI 32768
SF 300.1300049 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

1D NMR plot parameters
CX 20.00 cm
F1P 11.000 ppm
F1 3301.43 Hz
F2P -1.000 ppm
F2 -300.13 Hz
PPMCM 0.60000 ppm/cm
HZCM 180.07800 Hz/cm

(9)



(After column)

jk-1-D tbs deprotected 10/11/2000

Current Data Parameters
NAME ebh-II-4
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20001011
Time 14.53
INSTRUM spect
PROBHD 5 mm QNP 1H
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 26
DS 2
SWH 6172.839 Hz
FIDRES 0.094190 Hz
AQ 5.3084860 sec
RG 724.1
DW 81.000 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 11.00 usec
PL1 -1.00 dB
SFO1 300.1318534 MHz

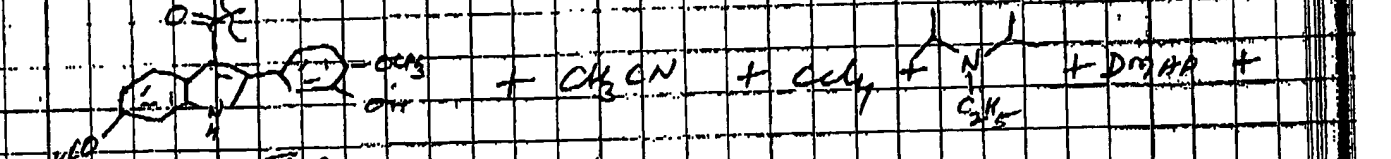
F2 - Processing parameters
SI 32768
SF 300.1300049 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

1D NMR plot parameters
CX 20.00 cm
F1P 9.000 ppm
F1 2701.17 Hz
F2P -0.500 ppm
F2 -150.07 Hz
PPMCM 0.47560 ppm/cm
HZCM 142.56175 Hz/cm

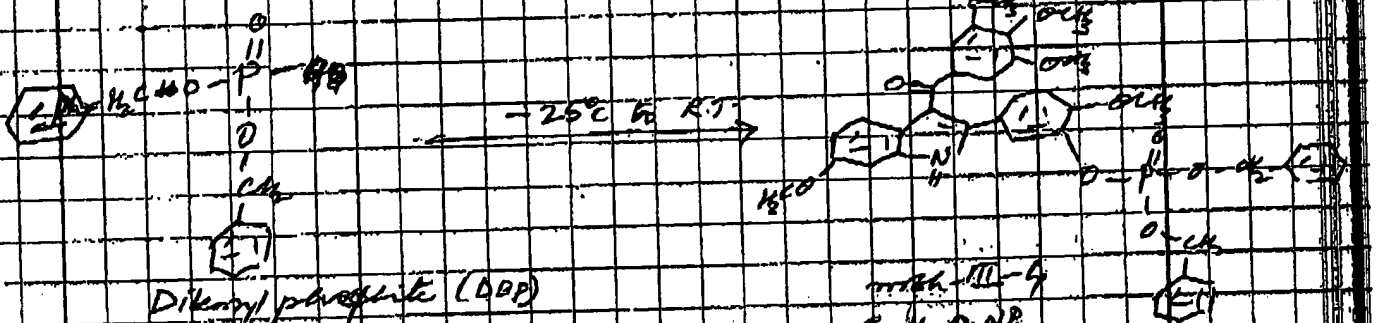
Exhibit 10

(see attached)

11/5/00



	mrbk-III-3	CCl_4	$C_8H_{11}N$	$C_4H_{10}N_2$
MF	$C_{16}H_{26}O_4N$			
MW	463	154.82	129.25	122
Ratio	1	8.8	2.1	0.1
Mols	0.2807 mmol	2.47	0.589	0.028
Gms	0.13 g	0.382g	0.076g	0.003g
		4.6mL	(d = 1.594 g/mL) ≈ 0.24mL	(d = 0.742 g/mL) ≈ 0.1mL



Dikenyl phosphite (DDP)

	mrbk-III-4
MF	$C_{14}H_{15}O_3P$
MW	262.25
Ratio	1.5
mols	0.4211
Gms	0.11 g
	$C_{40}H_{46}O_{10}N_2P_2$
	752.25

Ref: This thesis P. no. 45

Procedure: To a cay of mrbk-III-2 & well-stirred 50% g mrbk-III-2 acetonitrile at -25°C was added 0.24 mL g CCl_4. After stirring for about 15 mins diethylisopropyl amine and DMAP were added and stirred. after 10 mins. Dikenyl phosphite was added and the mixture was stirred at -20°C, for about 2 hrs. Then, the mixture was warmed to -10°C & R.T and stirred for another additional 2 hrs at R.T.

Work up: * About 10 mL g 0.5M KH_2PO_4 50% was added, and the product was extracted 2 EtOAc (3 × 20 mL)
* Washed the Organic Layer with brine and water.
* Dried over Na_2SO_4 and rotovaped.

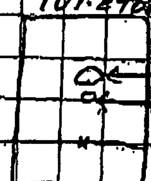
70% EtOAc soln

Product (yellow col. on TLC)
S1 → mh-III-3
S2 → Dibenzyl phosphite
R → Rxn. mixture

After about 3 hr stirring at R.T.

11/6/00 Did a column.
Loaded c̄ little EtOAc.
Hexanes, 10% EtOAc, 20% EtOAc, 30% EtOAc, 40% EtOAc, 50% EtOAc
50% → Nothing eluted
70% EtOAc ct → Got the pdt.

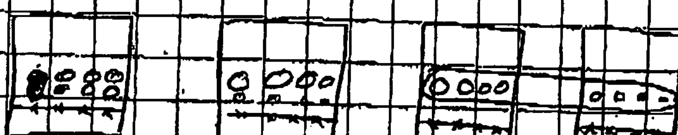
70% EtOAc



← Pdt.
← Unreacted mh-III-3

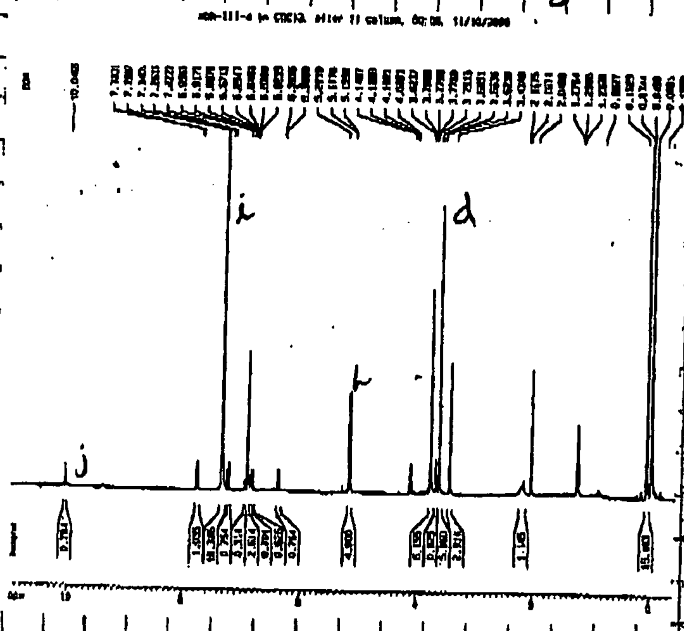
11/7/00 Took the NMR c̄ looked little messy so decided to do another column.

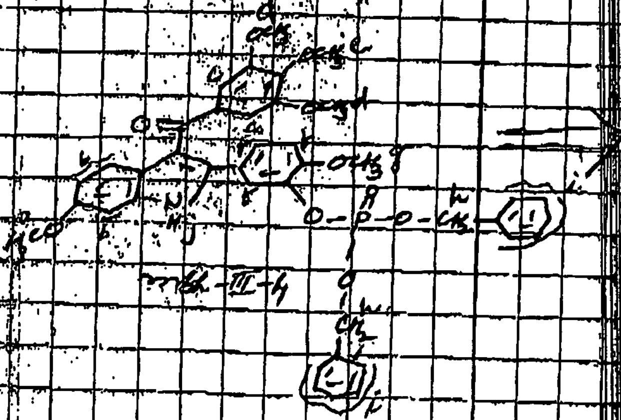
11/8/00 Did a second column.
→ Loaded the column c̄ EtOAc and eluted c̄ 50% EtOAc ...



— collected these and took NMR.

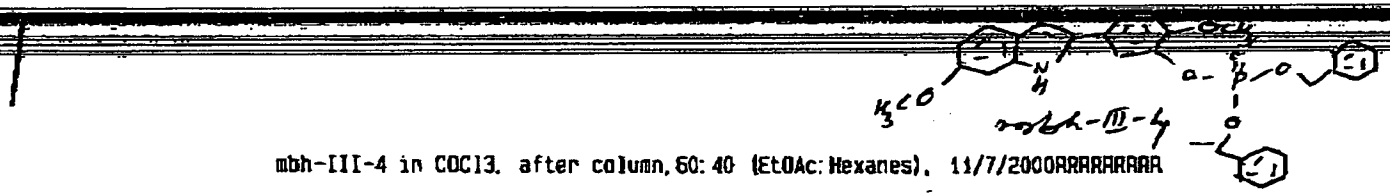
11/10/00 Took the NMR c̄ looked good and decided to go ahead.



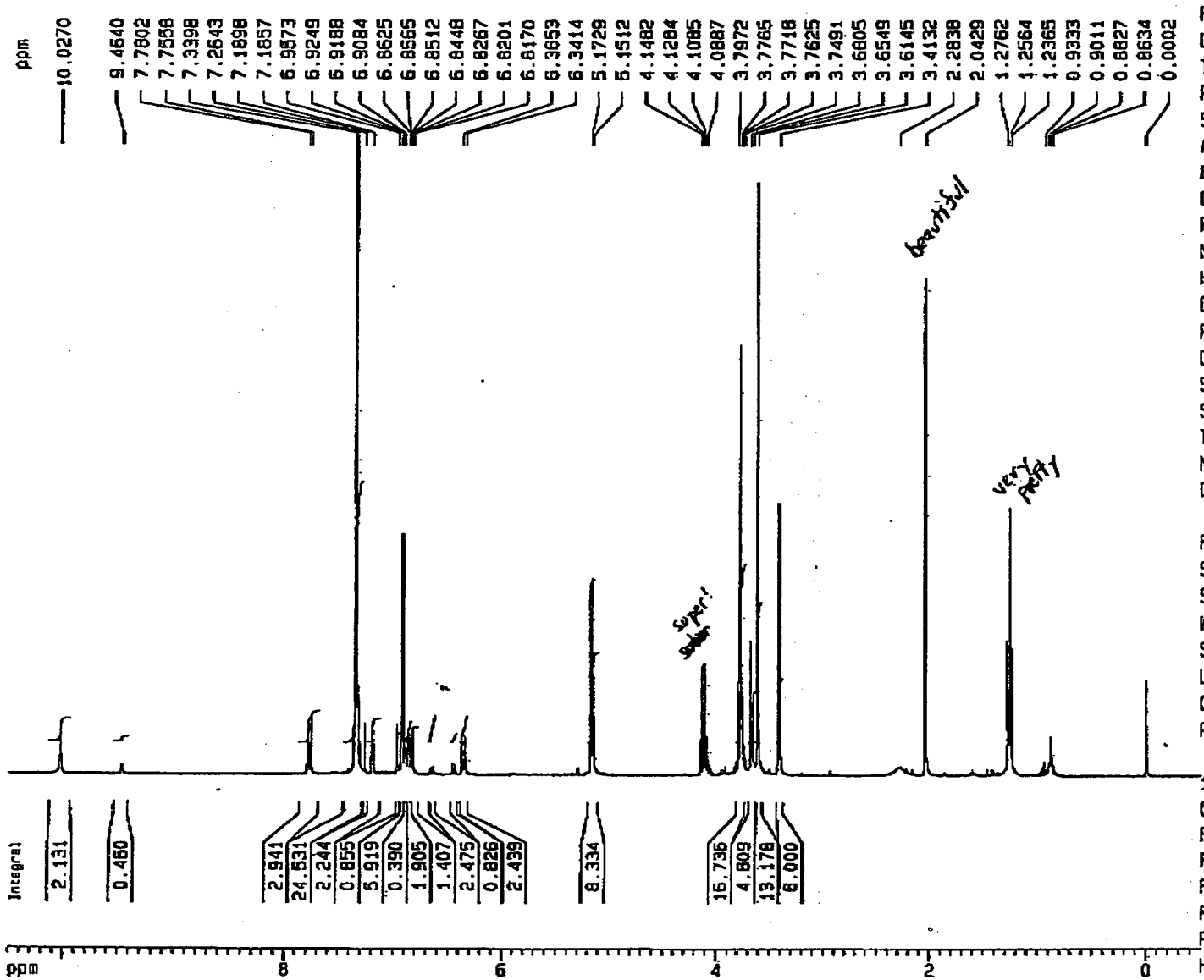


98

(82)



mbh-III-4 in COCl3. after column, 60: 40 (EtOAc: Hexanes). 11/7/2000RRRRRRRRR



Current Data Parameters

NAME	mbh14106
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

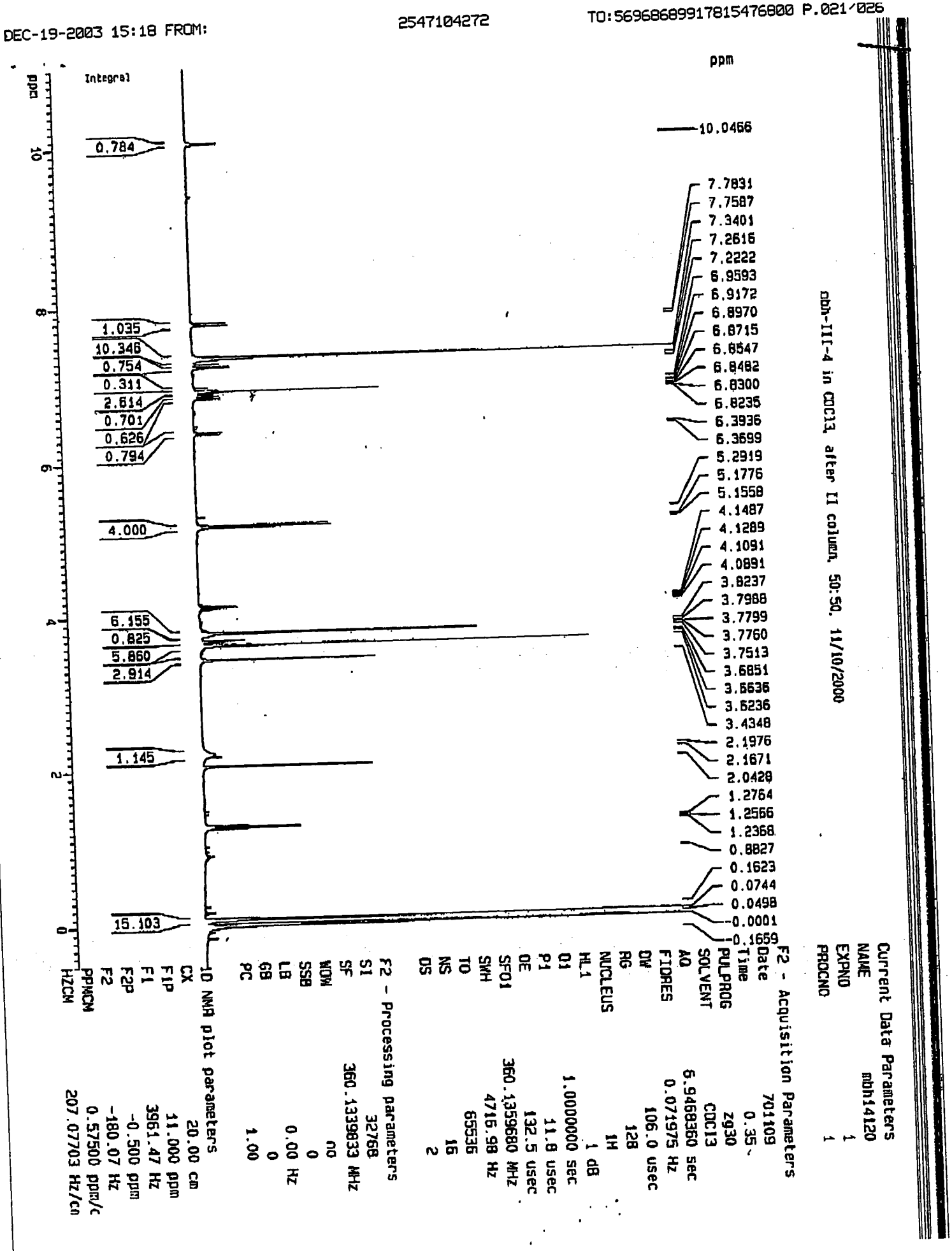
Date	701106
Time	10.31
PULPROG	zg30
SOLVENT	CDCl3
AQ	6.9468360 sec
FIDRES	0.071975 Hz
DW	106.0 usec
RG	128
NUCLEUS	1H
HL1	1 dB
D1	1.0000000 sec
P1	11.8 usec
DE	132.5 usec
SFO1	360.1359680 MHz
SWH	4716.98 Hz
TD	65536
NS	16
DS	2

F2 - Processing parameters

SI	32768
SF	360.1339820 MHz
WDW	no
SSB	0
LB	0.00 Hz
GB	0
PC	1.00

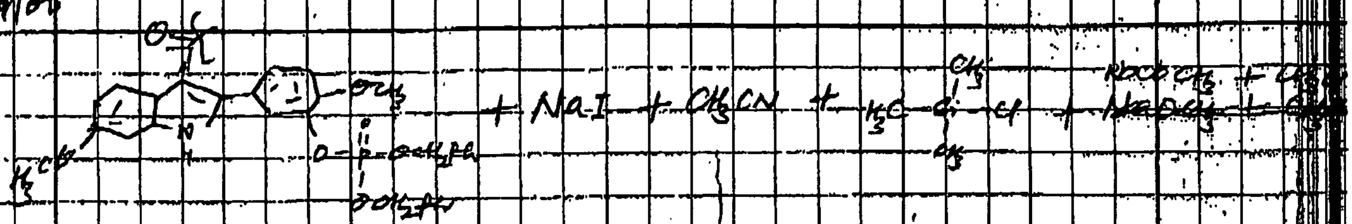
1D NMR plot parameters

CX	20.00 cm
F1P	10.500 ppm
F1	3781.41 Hz
F2P	-0.500 ppm
F2	-180.07 Hz
PPMCM	0.55000 ppm/c
HZCM	198.07368 Hz/cm

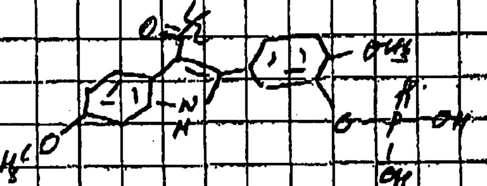

mbh-III-4 in CDCl3, after II column, 50:50, 11/10/2000
Current Data Parameters
NAME mbh14120
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date 701109
Time 0.35
PULPROG zg30
SOLVENT CDCl3
AQ 6.9468360 sec
FIDRES 0.071975 Hz
DW 106.0 usec
RG 128
NUCLEUS 1H
HL1 1 dB
O1 1.0000000 sec
P1 11.8 usec
OE 132.5 usec
SFO1 360.1359680 MHz
SWH 4716.98 Hz
TD 65536
NS 16
DS 2
F2 - Processing parameters
SI 32768
SF 360.1339833 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.00
10 NMR plot parameters
CX 20.00 cm
F1P 11.000 ppm
F1 3961.47 Hz
F2P -0.500 ppm
F2 -180.07 Hz
PPMCM 0.57500 ppm/c
HZCM 207.07703 Hz/cm
Integral
ppm
10.0466
7.7831
7.7587
7.3401
7.2615
7.2222
6.9593
6.9172
6.8970
6.8715
6.8547
6.8482
6.8300
6.8235
6.3936
6.3699
5.2919
5.1776
5.1558
4.1487
4.1289
4.1091
4.0891
3.8237
3.7988
3.7799
3.7760
3.7513
3.6851
3.6636
3.6236
3.4348
2.1976
2.1671
2.0428
1.2764
1.2566
1.2368
0.8827
0.1623
0.0744
0.0498
-0.0001
-0.1659
0.784
1.035
10.346
0.754
0.311
2.614
0.701
0.626
0.794
4.000
6.155
0.825
5.860
2.914
1.145
15.103

(34)

11/19/01



	mgh-III-4	~~NaI~~			
MF	$C_{40}H_{28}O_{10}NP$	~~NaI~~		SiH_9SiCl	CH_3ONa
MW	723.25	149.9		108.64	54
Ratio	1	2		2	2
mmole	0.076	0.152		0.152	0.152
Gm	0.055g	0.023g	1.5mL	0.0165g (d = 0.856g/mL ≈ 0.02mL)	0.0082g



+ ~~NaOMe~~ + ~~CH3OH~~

mgh-III-5a

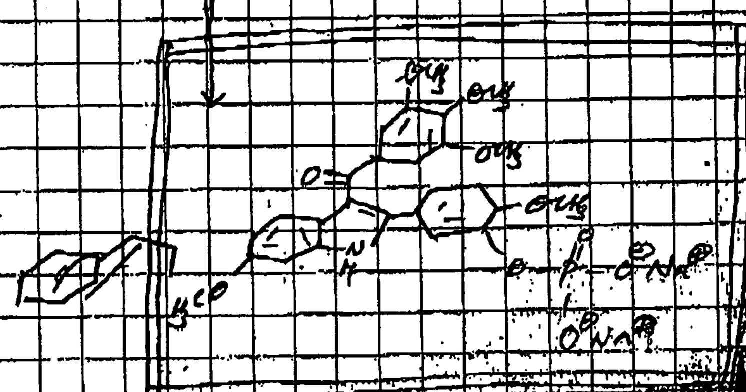
MF

MW

Ratio

mmole

Gm



MGH-III-5

$C_{36}H_{?}O_{10}NNa_2$ [illegible]

587.426

mp = 197 [illegible]

4*

Refi:- 'Anti-Cancer drug design' 1998, 13, 183-91 and Zhai thesis P. no. 45

Procedure: * To a well stirred sol* of mesh-III-4 and NaI in dry acetonitrile at R.T. was added Chlorotrimethyl silane and stirred at R.T. for 40 mins.

* Checked by TLC. * After adding TMS-Cl, the color of the reaction mixture was changed to orange

70% EtOAc

S → mesh-III-4
R → Rxn. mixture
* All the spots yellow in TLC

Pet.

After 40 mins

* water was added slowly followed by 5 mL of 10% Sodium thiosulfate sol* (to remove the yellowish-brown color)
* The organic phase was separated and the aqueous layer was extracted with EtOAc (4 x 10 mL)
* The combined organic layer is recovered to get white yellowish-white powder (mesh-III-5a), which was dissolved in 1.5 mL of dry methanol
* Then, 0.0082 g of NaOMe was added and stirred overnight at R.T.

11/12/00 Checked by both normal and reversed phase (RP) TLC, ⇒ no change

70% EtOAc

70:30 (MeOH:Isopropanol)

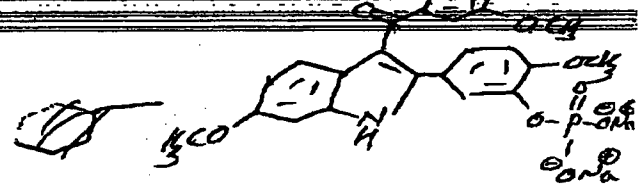
S R

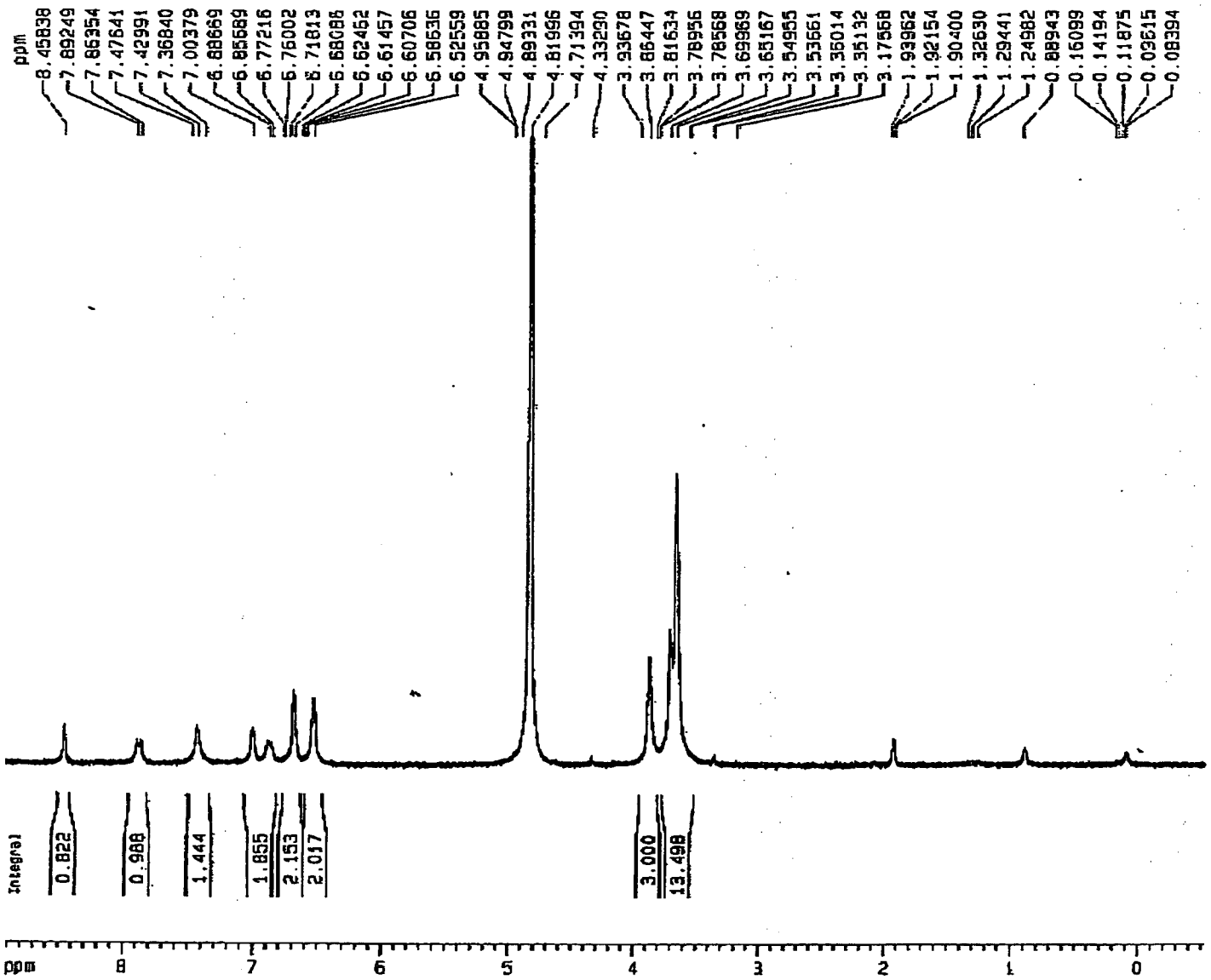
RP

* Continued stirring till evening and checked by TLC ⇒ No change
* Added one more equivalent of NaOMe and stirred over night.

11/13/00 Checked by TLC (RP) ⇒ No change
* Recovered methanol and recrystallized from methanol and water, and left in the refrigerator overnight.

mbh-II-23 in D2O, sample from the vial file, 07/05/2002





Current Data Parameters

NAME	mbh-II-23
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20020705
Time	23.25
INSTRUM	spect
PROBHD	5 mm QNP 1H/1
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	6172.839 Hz
FIDRES	0.094190 Hz
AQ	5.3084860 sec
RG	362
DW	81.000 usec
DE	6.00 usec
TE	300.0 K
D1	1.00000000 sec

======== CHANNEL f1 ========

NUC1	1H
P1	11.00 usec
PL1	-1.00 dB
SFO1	300.1318534 MHz

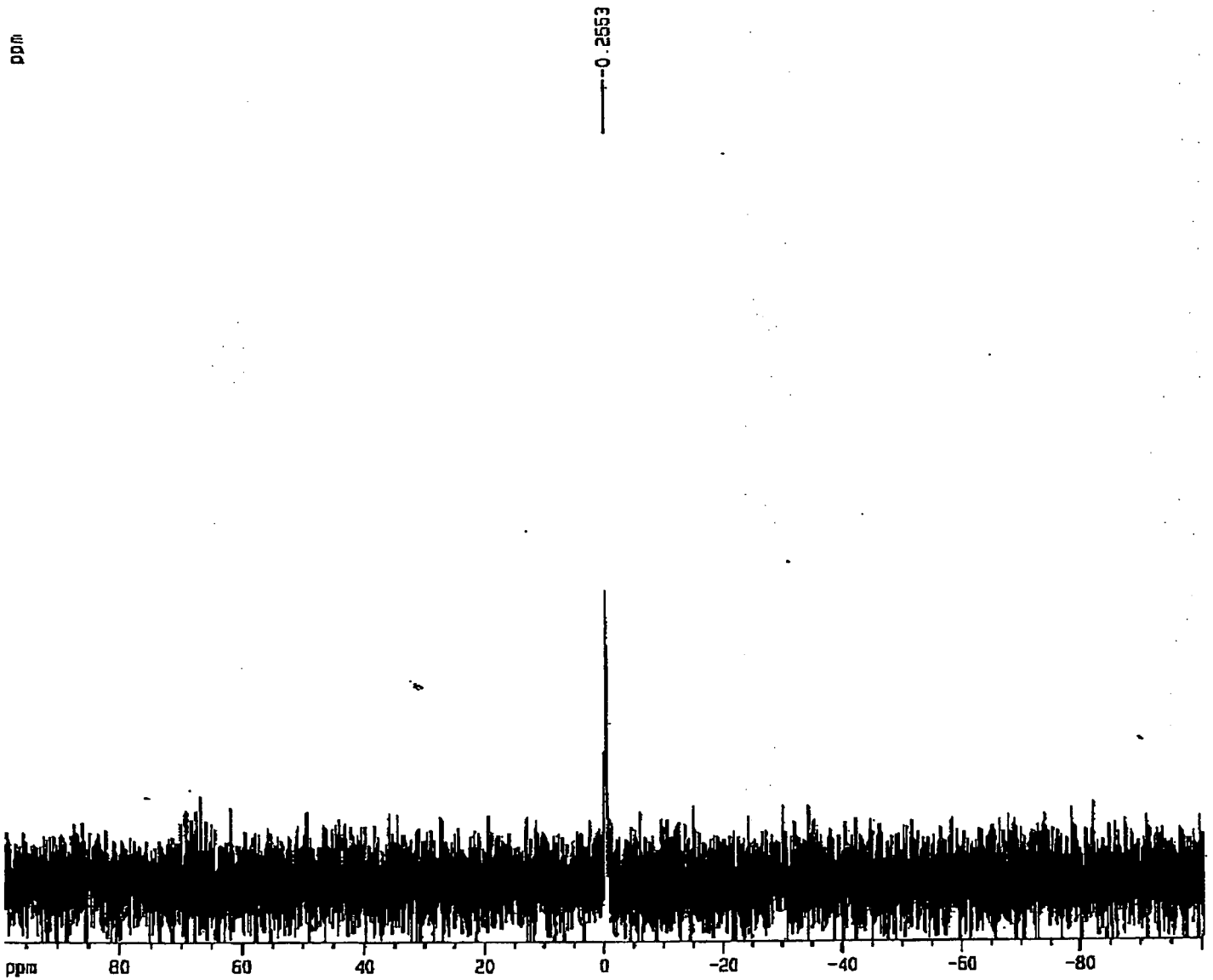
F2 - Processing parameters

SI	32768
SF	300.1299628 MHz
WDW	no
SSB	0
LB	0.00 Hz
GB	0
PC	1.00

1D NMR plot parameters

CX	20.00 cm
CY	16.89 cm
F1P	8.916 ppm
F1	2675.86 Hz
F2P	-0.524 ppm
F2	-157.36 Hz
PPMCM	0.47200 ppm/cm
HZCM	141.66122 Hz/cm

mbh-II-23 in D20. Sample from the vial file. 07/05/2002



Current Data Parameters
NAME mbh-II-23
EXPNO 4
PROCNO 1

F2 - Acquisition Parameters
Date_ 20020705
Time 23.46
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zgpg30
TD 65536
SOLVENT Acetone
NS 160
DS 4
SWH 48661.801 Hz
FIDRES 0.742520 Hz
AQ 0.6734324 sec
RG 7298.2
DW 10.275 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
d11 0.03000000 sec
d12 0.00002000 sec

CHANNEL f1
NUC1 31P
P1 8.50 usec
PL1 0.00 dB
SFO1 121.4886262 MHz

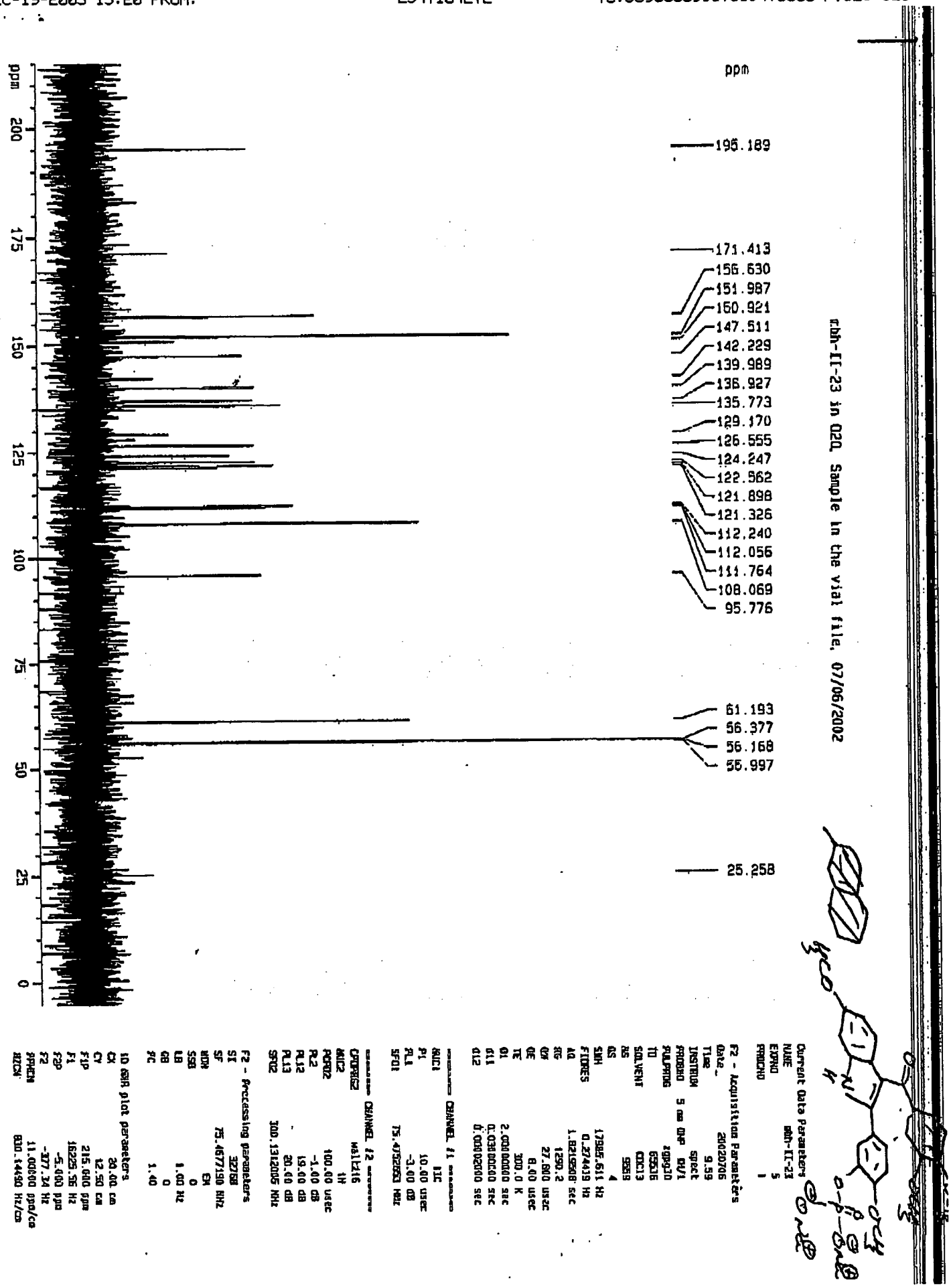
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 -1.00 dB
PL12 19.00 dB
PL13 20.00 dB
SFO2 300.1312005 MHz

F2 - Processing parameters
SI 32768
SF 121.4947410 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
CY 4.91 cm
F1P 98.448 ppm
F1 11960.92 Hz
F2P -100.117 ppm
F2 -12163.67 Hz
PPMCM 9.92825 ppm/cm
HZCM 1206.22986 Hz/cm

83

104


mbh-II-23 in CDCl, Sample in the vial file, 07/06/2002
ppm
195.189
171.413
156.630
151.987
150.921
147.511
142.229
139.989
136.927
135.773
129.170
126.555
124.247
122.562
121.898
121.326
112.240
112.056
111.764
108.069
95.776
61.193
56.377
56.168
55.997
25.258
Current Data Parameters
NAME mbh-II-23
EXPNO 5
PROCNO 1
F2 - Acquisition Parameters
Date_ 20020705
Time 9.59
INSTRUM spect
PROBHD 5 mm DUP QV/1
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 5888
DS 4
SWH 17985.611 Hz
FIDRES 0.274419 Hz
AQ 1.8219568 sec
RG 1290.2
DW 27.800 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
D11 0.03000000 sec
D12 0.00002000 sec
CHANNEL f1
NUC1 11C
P1 10.00 usec
PL1 -3.00 dB
SFO1 75.4752583 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 -1.00 dB
PL12 15.00 dB
PL13 20.00 dB
SFO2 300.1312005 MHz
F2 - Processing parameters
SI 32768
SF 75.4677190 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
CY 12.50 cm
F1P 215.000 ppm
F1 16225.56 Hz
F2P -5.000 ppm
F2 -377.34 Hz
PPMCM 11.00000 ppm/cm
HZCM 800.14490 Hz/cm
ppm
0
25
50
75
100
125
150
175
200